NJC

PAPER

View Article Online
View Journal | View Issue

Cite this: *New J. Chem.*, 2019, 43, 17046

Phenothiazine and amide-ornamented dihydropyridines *via* a molecular hybridization approach: design, synthesis, biological evaluation and molecular docking studies†

Ramar Sivaramakarthikeyan,[a] Shunmugam Iniyaval,[a] Krishnaraj Padmavathy,[a] Hui-Shan Liew,[b] Chin-King Looi,[b] Chun-Wai Mai[cd] and Chennan Ramalingan *[a]

Received 30th June 2019,
Accepted 8th October 2019

DOI: 10.1039/c9nj03394g

rsc.li/njc

A series of novel phenothiazinyldihydropyridine dicarboxamides **7a–7j** was synthesized by adopting a multi-step synthetic strategy and characterized through physical and spectral techniques. Among them, the chemical entities with *para*-fluoro (**7d**), *ortho*-bromo and -fluoro (**7f** and **7i**), *ortho*- and *para*-methyl (**7e**) and *meta*- and *para*-methoxy (**7h**) substituents exhibited either similar or superior anti-inflammatory activities with respect to the standard drug diclofenac sodium. Besides, the chemical entities with *ortho*-bromo and -fluoro substituents as well as *meta*-nitro substituents (**7f**, **7g** and **7i**) showed enhanced radical scavenging activities when compared to standard ascorbic acid. Furthermore, anticancer studies revealed that the *meta*- and *para*-chloro-substituted molecule **7a** exerted the best activity against all the pancreatic cancer cells tested. Also, appreciable binding affinity (−8.10 kcal mol^{-1}) was observed during molecular docking between B-cell lymphoma 2 and **7a**. The structural diversifications of the potent chemical entities besides further exploration in connection with the biological profiles of the same are underway.

Introduction

Cancer, a type of inflammation, is the second leading cause of death in the world. There were 9.6 million fatalities around the globe in 2018 due to life-threatening diseases and one in six was due to cancer. It is anticipated that the global death toll from cancer is increasing (approx. 12 million deaths by 2030). Specifically, the rate of death because of pancreatic cancer is found to be plausibly higher due to its highly invasive nature and chemoresistance.[1] It has been reported that in the United States, pancreatic cancer is the fourth leading cause of deaths from cancer.[2] Although various types of treatments are available for cancer, the treatments can cause one or more side effects. Numerous drugs are commercially available to treat cancers. Unfortunately, almost all of them are associated with serious side effects; consequently, it is a challenging task to save lives. The rising death rate every year because of cancer has made it obvious that there is an immediate need for the development of newer anticancer agents that can abolish cancer cells with no harm to regular tissues.

On the other hand, one of the vital targets of organic synthesis is a rapid assembly of diversified molecules, which is a key paradigm of the recent discovery of drugs; in this regard, multi-component reactions (MCRs) represent a useful approach to address this challenge. MCRs are nothing but chemical reactions between more than two reactants at the same time in one pot.[3] Several green chemistry principles are satisfied in these reactions and most of the atoms in each reactant are integrated into the eventual target. Furthermore, a one-pot reaction decreases the utilization of reagents and also solvents, especially those used for purification.[4] In addition to the aforementioned advantages, easy methodologies and equipment as well as their energy and time saving features have led to a reasonable number of efforts to design and implement MCRs not only in industries but also in academia.[5] The expediency of MCRs is even larger if these reactions offer "privileged biological structural motifs", which are structural components that are able to provide ligands for various functionalities, as well as structurally isolated receptors of biological importance, subsequently providing a platform to develop agents for various applications including pharmaceuticals.[6]

[a] *Department of Chemistry, School of Advanced Sciences, Kalasalingam Academy of Research and Education, (Deemed to be University), Krishnankoil, 626 126, Tamilnadu, India. E-mail: ramalinganc@gmail.com, c.ramalingan@klu.ac.in*
[b] *School of Postgraduate Studies, International Medical University, 57000, Kuala Lumpur, Malaysia*
[c] *Department of Pharmaceutical Chemistry, School of Pharmacy, International Medical University, 57000, Kuala Lumpur, Malaysia*
[d] *Centre for Cancer and Stem Cell Research, Institute for Research, Development and Innovation, International Medical University, 57000, Kuala Lumpur, Malaysia*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9nj03394g

Dihydropyridines, known as DHPs, embody a category of organic molecules of smaller size possessing a pyridine core, which is a vital structural component with a widespread biological profile.[7] Although five isomeric DHPs can exist theoretically, most of the documented ones possess either a 1,4-dihydro or 1,2-dihydro structure.[8] In particular, 1,4-DHPs have attracted much attention owing to their utility as calcium channel modulators with nifedipine, having a nitro functionality, besides the symmetrical substituents on its DHP possessing a first-generation derivative, as the first marketed calcium antagonist. The second-generation DHP drugs, namely, nitrendipine, felodipine, amlodipine, nilvadipine and nisoldipine, containing unsymmetrical substituents have also become products of commercial utility. While 1,4-DHPs have largely been produced as cardiovascular agents, a diverse range of DHPs have effectively been designed and used as various biological agents, including antitumor, vasodilators, antimycobacterial, and antihypertensive activity.[9]

Phenothiazines, a category of molecules possessing a structural motif fused with a thiazine and two-phenyl rings with a puckered conformation, have been recognized since the nineteenth century. In 1883, Bernthsen synthesized for the first time the parent molecule 10*H*-dibenzo-1,4-thiazine using elemental sulphur and diphenylamine.[10] Because of their attractive chemical characteristics as well as significant biological properties, their syntheses, structures, plausible reactions, properties and applications, especially with biological perspectives, were meticulously reviewed three decades ago and a retrosynthetic approach for the construction of phenothiazines was also depicted.[11,12]

In recent years, a large number of articles in connection with these molecules have been published and to date, more than 6000 derivatives of phenothiazine have been obtained. A minimum of eight to ten dozens of phenothiazine derivatives are utilized in treatment, largely as neuroleptics. One of the major advantages of this structural core is its ability to provide diversified derivatives, as substituents could effectively be introduced on the nitrogen at the 10-position, carbons at 1–4 as well as 6–9 positions and also oxidation could be made on sulphur so as to obtain sulfoxides and sulfones. In addition, the homoaryl rings could also be replaced by heteroaryl motifs, including pyrimidine, pyridine, quinoline, pyridazine, 1,2,4-triazine and pyrazine, to furnish the corresponding azaphenothiazines. Moreover, phenothiazine is reported to be non-toxic, which is unusual, and it is acceptable up to doses of the multi-gram scale in humans.[13] Chemical entities possessing the phenothiazine structural motif show a variety of pharmacological/biological properties, including antifungal, antibacterial, antitubercular, antiplasmin, anticancer, antioxidant, antiviral, anti-inflammatory, anticonvulsant, antipsychotic, antihistaminic, antitussive and anti-emetic. Their noteworthy properties were reviewed recently in several research papers, monographs and book chapters.[14–31]

Besides, the amide functionality is one of the most important structural units and can serve as a potential ligand component in the interaction between a molecule and host proteins *via* hydrogen bonding and/or van der Waals forces of attractions. Appropriate molecules incorporated with the amide functionality display various biological properties, including anticancer and antimalarial.[32–35] In our ongoing research programme searching for novel small organic molecules as potent biological agents, recently we were involved in the synthesis of phenothiazine/carbazole-integrated novel chemical entities as biological agents.[36–39] In particular, in one piece of our research, we synthesized a phenothiazine-tethered dihydropyrimidine analogue, which exhibited radical scavenging property of up to 69.34% (IC_{50} = 148 μM).[36] Further, we developed a few new series of molecules, specifically, acrylonitrile/acrylamide-integrated carbazoles/phenothiazines as well as hydrazide-integrated carbazoles and all the series exerted appreciable anticancer profiles.[37–39] In continuation of our research on the development of phenothiazine/carbazole-incorporated novel chemical entities as potent biological agents and also having the biological importance of the dihydropyridine and amide structural motifs in mind, a series of dihydropyridine, phenothiazine and amide hybridized novel chemical entities were designed and synthesized as biopertinent chemical entities. Described herein are the synthesis, characterization, anti-inflammatory, radical scavenging and anticancer evaluations as well as molecular docking studies of the novel hybrids 4-(10-alkyl-10*H*-phenothiazin-3-yl)-2,6-dimethyl-N^3,N^5-diaryl-1,4-dihydropyridine-3,5 dicarboxamides, as furnished in Fig. 1.

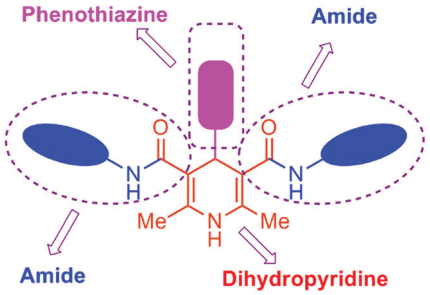
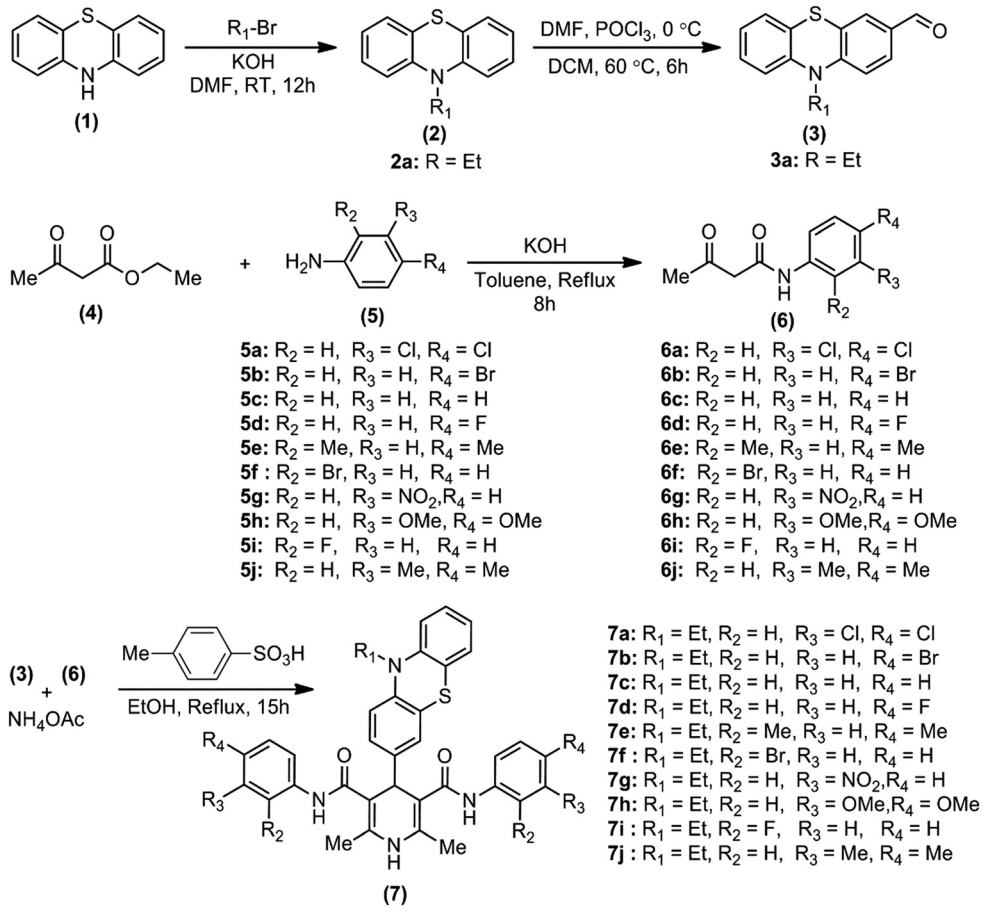


Fig. 1 Dihydropyridine, phenothiazine and amide hybridized novel chemical entities.

Results and discussion

Chemistry

The synthetic approach for the assembly of hybrid phenothiazinyldihydropyridine dicarboxamides **7a–7j** is furnished in Scheme 1. Initially, the active intermediate phenothiazine carbaldehyde **3a** was synthesized from the commercial phenothiazine **1** by adopting alkylation utilising bromoalkane in the presence of KOH in *N*,*N*-dimethyl formamide (DMF) followed by a Vilsmeier–Haack reaction using $POCl_3$ and DMF in dichloromethane.[40] On the other hand, the intermediates, 3-oxo-*N*-substituted phenylbutanamides **6a–6j** were synthesized from the reaction between the β-keto ester **4** and appropriately substituted anilines **5a–5j** in the presence of potassium hydroxide in toluene.[41] A three-component one-pot reaction between the phenothiazine carbaldehyde **3**, appropriate 3-oxo-*N*-substituted phenylbutanamides **6a–6j** and ammonium acetate in the ratio of 1 : 2 : 1.5, respectively, in the presence of *p*-toluenesulfonic acid in ethanol eventually produced the desired target hybrid

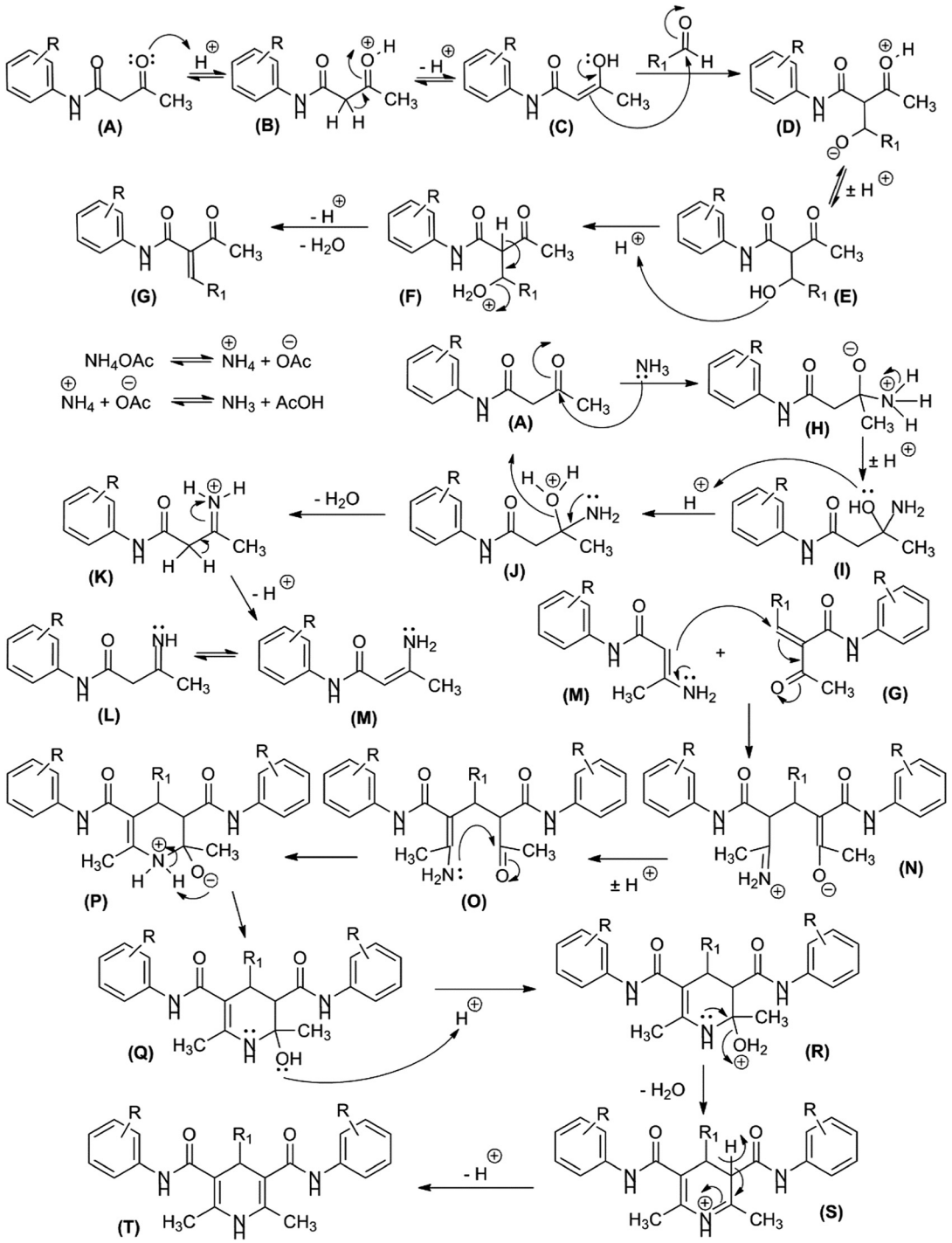


Scheme 1 Synthesis of hybrid phenothiazinyldihydropyridine dicarboxamides **7a–7j**.

phenothiazinyldihydropyridine dicarboxamides **7a–7j** in good to excellent yields. The structures of all the target molecules were unequivocally established based on physical and spectral methods.

In the ^{1}H NMR spectra of the target molecules **7a–7j**, the secondary amino proton of the dihydropyridine structural unit resonates between 5.0 and 6.0 ppm as a singlet with one proton integral, while the protons attached to the nitrogens of the amide moieties were observed between 8.2 and 10.0 ppm as a singlet with one proton integral. The protons of the methyl groups attached to the 2- and 6-positions of dihydropyridine core provide a singlet with six protons integral in the region 2.0 to 2.5 ppm, while the protons of the methyl group of ethyl moiety connected to phenothiazine resonate in the region 1.2 to 1.5 ppm as a three protons signal. The methylene protons of the ethyl group integrated at the nitrogen of the phenothiazine were observed in the region between 3.7 and 4.0 ppm as either a broad doublet or quartet with two protons integral. Further, the methine proton tethered at the 4-position of dihydropyridine resonates in the region 4.5 to 4.8 ppm as a one proton singlet, while the aromatic protons of the targets exhibit a multiplet signal in the area between 6.5 and 8.0 ppm. Besides, the protons of the methyl moieties connected to the aromatic ring in **7e** provide signals between 2.1 and 2.3 ppm as singlets with each of the six protons integral, while the ones connected to the aromatic moiety in **7j** give signals at nearly 2.3 ppm as singlets with each of the six protons integral. Eventually, the protons of the methoxy groups attached to the aromatic moiety in **7h** resonate as six protons each as singlets at nearly 3.8 ppm.

In the ^{13}C NMR spectra of the target chemical entities **7a–7j**, signals for the methyl carbons attached at the 2- and 6-positions of dihydropyridine structural motif could be observed in the region between 18.0 and 20.0 ppm. The methyl carbons of the ethyl group as well as methylene carbons of the ethyl group connected to the nitrogen of phenothiazine moiety resonate in the regions 12.0–13.0 ppm and 41.0–43.0 ppm, respectively. The methine carbons situated at the 4-position of dihydropyridine exhibit signals in the region between 40.0 ppm and 42.0 ppm while the carbons located at the 3- and 5-positions of the dihydropyridine core resonate in the region 103.0–107.0 ppm. Further, the carbons present at the 2- and 6-positions of the dihydropyridine could be observed in the regions 144.0–146.0 and 143.0–145.0 ppm. The carbonyl carbon of amide functionality exhibit signals in the extreme down-field region. In addition, the carbons of two methyl groups attached at the 2- and 4-positions of the phenyl rings in **7e** provide signals between 17.0 and 21.0 ppm, while the same integrated at the 3- and 4-positions of the phenyl rings **7j** offer signals between



Scheme 2 General mechanism for the synthesis of the phenothiazinyldihydropyridine dicarboxamides.

20.0 ppm and 21.0 ppm. In addition, the carbons of two methoxy groups located at the 3- and 4-positions of the phenyl rings in **7h** resonate between 54.0 ppm and 55.0 ppm. Besides, the signals for all the aromatic carbons were observed in the region between 115.0 ppm and 142.0 ppm. All these characteristics in addition to IR and microanalysis confirmed the target molecules.

The schematic representation of the plausible mechanistic pathway for the synthesis of the target dihydropyridines is furnished in Scheme 2. Here, the reactant amide **A** undergoes keto–enol tautomerism (**C**) in the presence of acid. Nucleophilic attack of the enolic bond on the carbonyl carbon of the aldehyde provides the secondary alcoholic intermediate **E** *via* **D**, which upon abstraction of a proton followed by the elimination of water gives the olefinic intermediate **G**. On the other hand, ammonium acetate is in equilibrium with its dissociated ammonium and acetate ions, which further is in equilibration

with ammonia and acetic acid. Nucleophilic attack of ammonia on the carbonyl carbon of the keto group of another molecule of amide **A** furnishes the intermediate **H**, which upon the addition and elimination of a proton yields the amino alcohol intermediate **I**. The addition of a proton followed by dehydration on **I** provides the cationic intermediate **K** through **J**, which upon elimination of a proton yields **M**, equilibrated with **L**. The olefinic bond of **M** then attacks one of the oleinic carbons of **G** during electronic shifts followed by proton abstraction and elimination, yielding a primary amino group possessing the intermediate **O** through **N**. The lone pair of electrons on the primary amino group (*i.e.* the nucleophile) then attack the carbonyl carbon of the intermediate as shown in **O** followed by electron shifts and proton transfer to provide the hydroxyl possessing intermediate **Q** through **P**. Proton abstraction followed by dehydration and deprotonation eventually furnish the target dihydropyridine **T** through the ammonium intermediate **S**.

Biological activities

Anti-inflammatory activity

The anti-inflammatory activity (*in vitro*) of all the synthesized chemical entities **7a–7j** was evaluated using the protein denaturation technique.[42,43] Protein denaturation is a methodology wherein the secondary structure and tertiary structure of a protein would be lost when an external stress or a molecule is applied. When denatured, the biological function of most biological proteins would be lost. A well-documented basis of inflammation is the denaturation of proteins. As part of the present investigation, the ability of the target molecules **7a–7j** to inhibit protein denaturation was evaluated. Diclofenac sodium was used as a standard drug, which exhibited 90% inhibition of protein denaturation. The percentage inhibitions of protein denaturation upon the utilization of the chemical entities **7a–7j** are provided with respect to the inhibition of protein denaturation by the standard and the results are furnished in Table 1 and Fig. 2.

Here, a phenothiazine and amide-ornamented dihydropyridine possessing an electron-releasing ethyl moiety on the nitrogen of the phenothiazine and electron-withdrawing chloro moieties on the *meta*- and *para*-positions of the aryl groups of the amide units (**7a**) was used, and it exhibited 78% inhibition when compared to the inhibition from the standard drug. Replacing *meta*-chloro substituents by hydrogens and *para*-chloro substituents by

Table 1 Anti-inflammatory activities of **7a–7j**

S. no.	Compound	Inhibition (%)
1	**7a**	78.44 ± 0.73
2	**7b**	71.58 ± 0.92
3	**7c**	96.11 ± 1.39
4	**7d**	103.90 ± 0.88
5	**7e**	100.76 ± 0.54
6	**7f**	100.63 ± 0.97
7	**7g**	85.59 ± 0.70
8	**7h**	103.75 ± 1.25
9	**7i**	105.69 ± 1.13
10	**7j**	95.78 ± 1.52

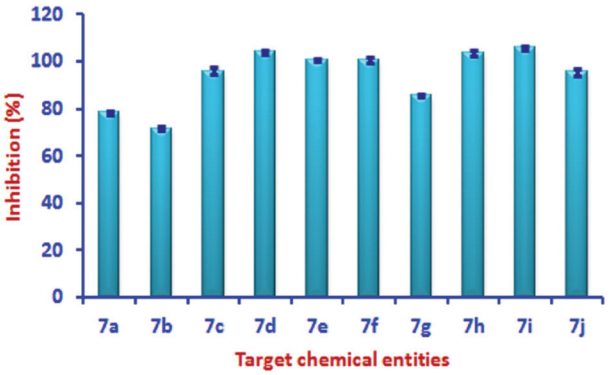


Fig. 2 Anti-inflammatory activity with respect to diclofenac sodium.

comparatively less electronegative bromo moieties in **7a** (*i.e.* **7b**) reduced the inhibition by 7%, while substituting a chloro group at *meta*-position by an electron-withdrawing nitro group and chloro group at the *para*-position by hydrogen in **7a** (*i.e.* in **7g**) increased the inhibition by 7%. Substituting electron-withdrawing chloro units on both the *meta*- and *para*-positions by hydrogens in **7a** (*i.e.* **7c**) improved the inhibition by 18%, while the introduction of electron-releasing methyl substituents on the *meta*- and *para*-positions of the aryl groups in place of the hydrogens in **7c** (*i.e.* **7j**) also exhibited an almost similar inhibition to that of **7c**. Further, the incorporation of a more electronegative fluoro substituent on the *para*-positions (**7d**), electron-releasing methyl substituents on the *ortho*- and *para*-positions (**7e**), electron-withdrawing bromo substituents on the *ortho*-positions (**7f**), electron-releasing methoxy substituents on the *meta*- and *para*-positions (**7h**) and more electronegative fluoro substituents on the *ortho*-positions (**7i**) of the aryl moieties of the amide scaffold furnished superior inhibition profiles and the results were equal and even slightly higher than that of the standard diclofenac sodium. Among all the chemical entities tested, the one possessing more electronegative fluoro functionality on the *ortho*-positions of the aryl units of the amide scaffolds (**7i**) furnished the best inhibition activity. Since the electronic effect of the substituents does not follow systematically with respect to the inhibition activity, a combination of two or more effects, including inductive, mesomeric and steric/field effects, might have played a role in proving the superior anti-inflammatory activity.

Radical scavenging activity

Reactive oxygen species (ROS) cause diverse neurodegenerative diseases, including Alzheimer's, autoimmune disorder, ulcer, mutagenesis and carcinogenesis, by hindering the function of numerous cellular components.[44,45] Antioxidants are nothing but agents that scavenge the free radicals formed in our biological system. Although a plethora of radical scavenging agents are available in nature, it is highly essential to develop a drugs with better antioxidant profiles to meet global needs. To determine the radical scavenging activity, a wide variety of *in vitro* methods, including a hydroxyl radical scavenging assay, ferric reducing antioxidant power and organic radical scavenging assay, are available. Of all the methods, organic radical scavenging by

Table 2 Radical scavenging of the target molecules **7a–7j**

Entry	Compound	% scavenging	IC_{50} (μM)
1	**7a**	90.32 ± 0.80	230.6
2	**7b**	61.18 ± 1.17	—
3	**7c**	100.12 ± 0.51	75.93
4	**7d**	85.70 ± 1.25	16.02
5	**7e**	63.42 ± 0.49	32.45
6	**7f**	110.62 ± 0.99	7.07
7	**7g**	110.45 ± 1.36	202.3
8	**7h**	51.87 ± 1.20	28.33
9	**7i**	108.78 ± 0.91	57.06
10	**7j**	43.56 ± 0.84	162.6

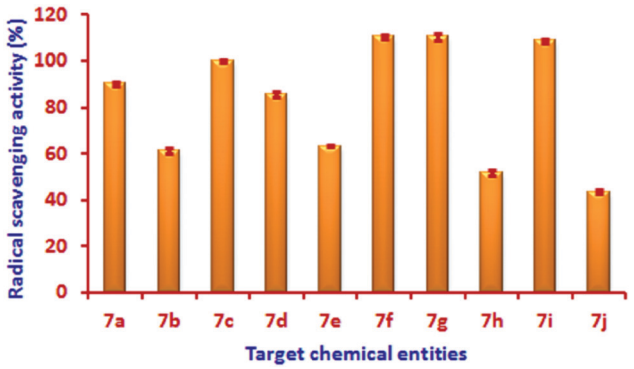


Fig. 3 Radical scavenging activity with respect to ascorbic acid.

DPPH is being widely used by researchers as it is the simplest one. Consequently, all the target phenothiazine and amide-tethered dihydropyridines **7a–7j** were evaluated for their radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH), a reactive oxygen species.[46] The activity was estimated in terms of DPPH inhibition and the outcomes are furnished in Table 2 and Fig. 3 with respect to the activity of ascorbic acid, a standard one.

The phenothiazine and amide-tethered dihydropyridine possessing electron-withdrawing chloro moieties on the *meta*- and *para*-positions of the aryl groups of the amide structural motif (**7a**) exhibited 90% inhibition of DPPH radicals when compared with the inhibition by the standard ascorbic acid. Integration of a hydrogen and comparatively less electronegative bromine on the *meta*- and *para*-positions, respectively, in place of chlorine present on the aryl groups (**7b**) reduced the activity by nearly 30%. Substituting electron-releasing methyl moieties on the *ortho*- and *para*-positions of the aryl units of the amide scaffold (**7e**) furnished no significant improvement in the inhibition when compared with **7b** containing an electron-withdrawing unit. Further, integration of electron-releasing methoxy substituents on the *meta*- and *para*-positions of the aryl units as well as electron-releasing methyl substituents on the same (**7h** and **7j**, respectively) reduced the radical scavenging activity by nearly 50% when compared with the standard. Besides, the incorporation of the more electronegative fluoro functionality on the *para*-positions of the aryl units (**7d**) provided comparable activity with that of **7a** though slightly lower. However, the chemical entity with no substituents on the aryl moieties of the amide scaffold (**7c**) furnished similar activity with that of the standard, while the molecules with electron-withdrawing bromo-, nitro- and fluoro-substituents on the *ortho*-, *meta*- and *ortho*-positions, respectively, of the aryl part of the amide scaffold (**7f**, **7g** and **7i**, respectively) exerted superior activity when compared with the standard ascorbic acid. Since the activity variations do not depend on any particular factors, such as electronic, mesomeric and steric/field effects, the overall activity may be a result of two or more combinations of the same.

Anticancer activity

Cancer is one of the deadliest diseases in the world and there are several types of cancers that affect humans. Although numerous types of drugs are available in the market to treat cancers, the development of newer drugs in connection with the same is essential as there are no specific drugs that can yet completely cure the cancers, and also the drugs, in general, tend to become resistant over a period of time due to biological/environmental changes. In the present study, cytotoxicity studies of all the synthesized phenothiazine and amide-tethered dihydropyridines **7a–7j** were evaluated against the pancreatic cancer cell lines SW1990, AsPC1, BxPC3, Panc1 and the non-cancerous cell line MRC5 by the Cell-Titre Glo Luminescent Cell Viability Assay method. Against the tested target molecules, the percentages of pancreatic cancer cells survival are displayed in Table 3.

Molecular docking

The molecular origins of the interactions among the BCL-2 (B-Cell Lymphoma 2) active sites and the binding affinity, taken

Table 3 Anticancer activity of the target chemical entities **7a–7j**

		IC_{50} (μM)				
Entry	Compound	SW1990	AsPC1	BxPC3	Panc1	MRC5
1	**7a**	8.63 ± 1.07	7.47 ± 0.73	6.31 ± 1.00	7.39 ± 0.03	70.51
2	**7b**	>100	>100	16.91 ± 0.24	22.87 ± 0.11	>100
3	**7c**	92.80 ± 6.26	61.53 ± 3.94	54.07 ± 6.71	50.12 ± 3.19	>100
4	**7d**	30.99 ± 0.17	30.12 ± 0.08	30.76 ± 0.60	30.26 ± 1.04	47.05 ± 0.45
5	**7e**	20.83 ± 2.24	25.05 ± 9.03	15.93 ± 0.81	19.37 ± 1.77	22.15 ± 0.80
6	**7f**	26.43 ± 4.89	18.71 ± 1.95	28.48 ± 1.24	29.29 ± 1.16	45.22 ± 2.04
7	**7g**	18.00 ± 0.35	15.20 ± 0.42	15.01 ± 0.32	12.16 ± 0.09	22.42 ± 1.56
8	**7h**	24.48 ± 9.90	18.69 ± 0.87	17.36 ± 0.13	18.79 ± 0.37	16.72 ± 2.31
9	**7i**	>100	89.07 ± 4.62	59.93 ± 2.80	26.72 ± 2.21	>100
10	**7j**	18.83 ± 1.92	18.82 ± 0.86	11.83 ± 1.94	19.48 ± 0.11	21.35 ± 0.41

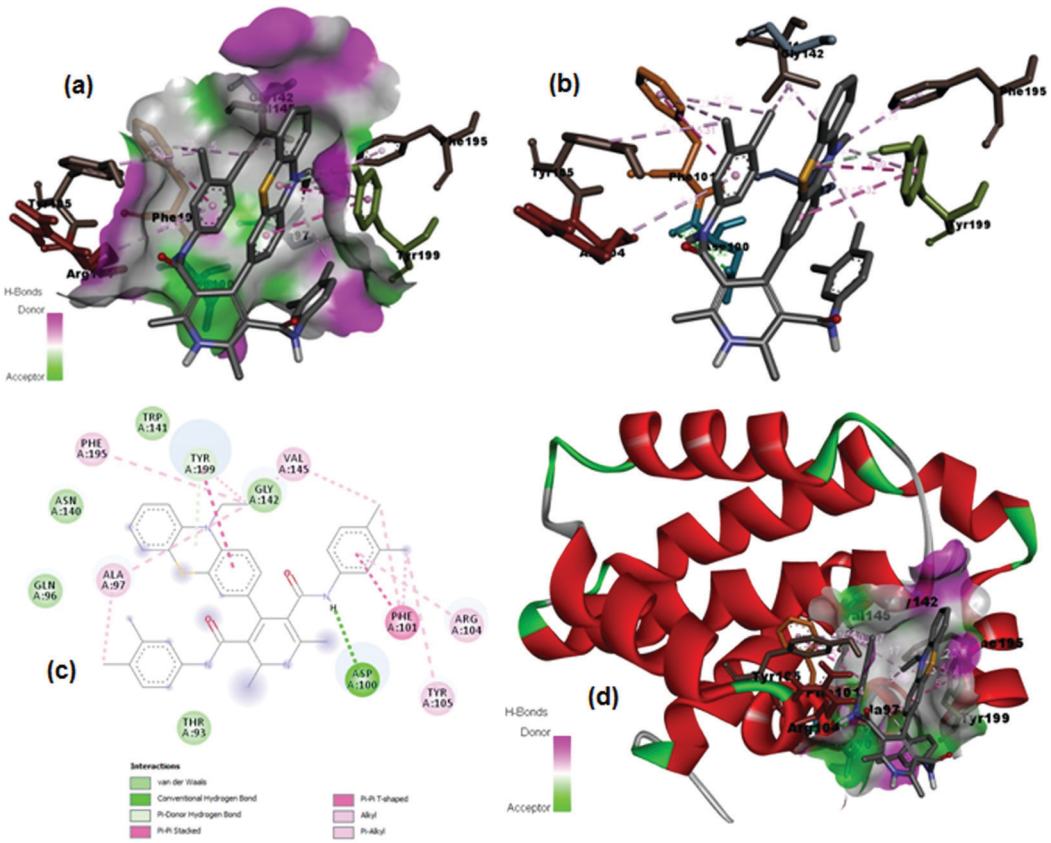


Fig. 4 2D and 3D interactions of BCL-2 with **7a**.

from the protein data bank, and the structure of **7a**, a conformationally stabilized one, were evaluated through molecular docking studies by utilizing Auto dock version 4.0. The outputs obtained through docking are provided in Fig. 4.

The phenothiazine and amide-tethered dihydropyridine **7a** showed a binding energy of -8.10 kcal mol^{-1} along with a predicted IC_{50} value of 1.16 μM. The molecule **7a** possessed van der Waals interaction with two polar hydrophilic amino acids, namely glutamine and asparagine, and one non-polar amino acid, namely threonine, as well as two non-polar hydrophobic amino acids, namely tryptophan and glycine. Additionally, a conventional hydrogen bond interaction between aspartic acid, an electrically charged acidic amino acid, and a hydrogen of the secondary amino functionality of the amide unit was noted. A π-donor hydrogen bond interaction between tyrosine, a polar hydrophilic amino acid, and one of the phenyl rings of the phenothiazine structural unit was also observed. Besides, there existed an alkyl–alkyl interaction between a non-polar hydrophobic amino acid, namely glycine, and an alkyl group of phenothiazine. Further, there were three π-alkyl interactions between the alkyl group present in the phenothiazine structural unit and non-polar hydrophobic amino acids, such as phenyl alanine, valine and alanine, observed. In addition, a couple more π-halo interactions, such as an interaction between a polar hydrophilic amino acid, tyrosine and a chloro group connected to one of the aryl units of the amide scaffold, as well as an interaction between an electrically charged basic amino acid arginine and one of the aryl rings of the amide structural motif were also noticed.

Conclusion

A series of phenothiazine and amide-tethered dihydropyridines **7a–7j** were synthesized through a multi-step synthetic methodology and characterized using physical and spectral methods. Anti-inflammatory studies revealed that the molecules incorporated with fluoro substituent on the *para*-position (**7d**), bromo and fluoro substituents on the *ortho*-positions (**7f** and **7i**), methyl substituents on the *ortho*- and *para*-positions (**7e**) as well as methoxy substituents on the *meta*- and *para*-positions (**7h**) showed either a similar or superior inhibition profile in comparison with the inhibition profile of the standard anti-inflammatory drug diclofenac sodium. Further, the radical scavenging activity studies implied that the molecules possessing bromo and fluoro substituents on the *ortho*-position of the aryl moieties of the amide scaffolds as well as the nitro substituent on the *meta*-position of the aryl moieties of the amide structural unit (**7f**, **7g** and **7i**) exhibited superior activity when compared to the standard ascorbic acid. Besides, the study of their anticancer activity exposed that among the target molecules, the chemical entity incorporated with *meta*- and *para*-chloro substituents on the aryl ring of the amide structural motif (**7a**) exerted superior activity against all the human pancreatic cancer

cell lines. In addition, molecular docking between B-cell lymphoma 2 (BCL-2) with **7a** furnished a high binding affinity (-8.10 kcal mol^{-1}). Further exploration of the anti-inflammatory, radical scavenging and anticancer activities of the existing respective potent molecules, besides structural diversification of the selected molecules that offered better results in order to develop novel drugs, are presently under progress.

Experimental

General

All the chemicals were purchased from commercial sources as reagent grade and used as received. By employing standard procedures, all the solvents were dried/distilled prior to their utilization. Pre-coated (silica-gel) TLC sheets were utilized for TLC and visualized by long- and short-wavelength UV lamps. Column chromatography was performed on silica gel (100–200 mesh, spherical) slurry packed in glass columns. The eluent systems used for individual separations are furnished in the respective experimental procedures. All the FT-IR spectra in KBr pellets were recorded on a Shimadzu IR Tracer-100 spectrophotometer in the range of 4000–400 cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on an NMR spectrometer (Bruker AVANCE II 400 and 100 MHz) at 25 °C with the use of tetramethylsilane (TMS) as an internal standard and $CDCl_3$/DMSO-d_6 as the solvent and the chemical shifts are expressed herein in terms of parts per million (δ ppm). The spin multiplicities s, d, t, q and m stand for singlet, doublet, triplet, quartet and multiplet, respectively.

Synthesis of ethyl phenothiazine 2. In a typical experiment, a round-bottom flask (250 mL) was charged with 10*H*-phenothiazine (0.025 mol) and DMF (50 mL). Powdered KOH (0.03 mol) was then added into the flask and it was stirred at ambient temperature for 2 h. To this solution, bromoethane (0.04 mol) was added in a drop-wise fashion through a pressure equalizer for 20 min and the reaction was continued for 24 h at the same temperature. The progress of the reaction was monitored through TLC. The reaction mixture was then extracted with dichloromethane (3 × 20 mL), washed well with water and dried over anhydrous sodium sulphate. Evaporation of the dichloromethane followed by purification through column chromatography (5% ethyl acetate in hexane) eventually yielded the title compound **2**.[40]

Synthesis of carbaldehyde 3. In a two-neck round-bottom flask (100 mL), DMF (1 mL) was charged under an inert atmosphere. $POCl_3$ (1.2 mL) was then added in a drop-wise fashion at 0 °C and stirred for 45 min. Phenothiazine **2** (11.0 mmol) in chloroform (20 mL) was then added drop-wise into the reaction mixture. After completion of the addition, the temperature was raised to 60 °C and the stirring was continued for 6 h. The completion of the reaction was monitored through TLC. It was then extracted with dichloromethane (3 × 20 mL), washed well with water, dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The reaction mixture thus obtained was subjected to column chromatography (15–20% ethyl acetate in hexane) to yield the pure product **3**.[40]

General method for the synthesis of 3-oxo-*N*-substituted phenylbutanamides (6a–6j). To a solution of ethylacetoacetate (2 g, 15.4 mmol) in toluene (20 mL) was added powdered KOH (15 mol%). The respective substituted anilines (17.0 mmol) were then successively added to the solution. The reaction mixture was heated so as to reflux and this was continued for 8 h under continuous stirring. The mixture was extracted with dichloromethane after attaining room temperature, and washed with water. The organic layer thus obtained was dried over anhydrous sodium sulphate and evaporated. The crude mass thus resulted was subjected to silica-gel column chromatography utilizing 10–20% ethyl acetate in hexane to eventually yield the respective substituted phenylbutanamides **6a–6j**.[41]

General method for the synthesis of the target hybrid molecules 7a–7j. To a mixture of carbaldehyde **3** (1 equiv.), the respective substituted phenyloxobutanamides **6a–6j** (2 equiv.) and ammonium acetate (1.5 equiv.) in ethanol (15 mL) under reflux conditions, was added *p*-toluenesulfonic acid (15 mol%). The refluxing was continued for 12–15 h while the progress was monitored by TLC. After completion of the reaction, the content of the flask was cooled to ambient temperature and the solvent was removed under reduced pressure. The crude obtained was then extracted with dichloromethane after diluting with water. The organic portion was separated, dried on anhydrous sodium sulphate and evaporated under reduced pressure. The solid thus obtained was subjected to column chromatography utilizing 30–50% ethyl acetate in hexane as the eluent to afford the respective target hybrid molecules **7a–7j** in pure form as solids.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7a. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6a** (0.97 g, 3.92 mmol) and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) furnished the target hybrid phenothiazinyldihydropyridinedicarboxamide **7a**. Yield, 1.18 g (85%); MP: 250–252 °C; FT-IR (KBr, cm^{-1}): ν 3645.4, 3230.7, 3091.8, 2922.1, 2850.7, 1699.2, 1670.3, 1581.6, 1508.3, 1465.9, 1375.2, 1323.1, 1234.4, 1130.2, 1109.0, 1085.9, 1026.1, 875.6, 813.9, 746.4, 675.0, 644.2, 572.8, 509.2, 484.1, 466.7, 429.7; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (s, 2H), 7.59–6.77 (m, 13H), 5.74 (s, 1H), 4.83 (s, 1H), 3.86 (br.d, J = 6.60 Hz, 2H), 2.29 (s, 6H), 1.36 (t, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.6, 144.8, 144.5, 139.9, 138.7, 136.2, 132.3, 128.1, 127.4, 127.3, 126.7, 126.6, 125.6, 124.9, 124.0, 122.6, 122.5, 122.4, 115.7, 115.0, 113.8, 106.4, 41.8, 41.4, 19.2, 13.0; anal. calcd for $C_{35}H_{28}C_{l4}N_4O_2S$ (%): C, 59.17; H, 3.97; N, 7.89; S, 4.51; found: C, 59.38; H, 3.91; N, 7.95; S, 4.44.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7b. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6b** (1.0 g, 3.92 mmol), and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) yielded the target hybrid phenothiazinyldihydropyridinedicarboxamide **7b**. Yield, 1.27 g (88%); MP: 146–148 °C; FT-IR (KBr, cm^{-1}): ν 3793.9, 3664.7, 3591.4, 3140.1, 3047.5, 2357.0,

2322.2, 1651.0, 1597.0, 1504.4, 1354.0, 1330.8, 1228.6, 1195.8, 1010.7, 1066.6, 958.6, 835.1, 756.1, 731.0, 688.5, 615.2, 580.5, 524.6, 424.3; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (2s, 2H), 7.65–6.77 (m, 15H), 5.77 (s, 1H), 4.63 (s, 1H), 3.87 (d, J = 6.80 Hz, 2H), 2.16 (s, 6H), 1.37 (t, J = 7.00 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.9, 144.8, 144.4, 139.9, 138.8, 136.1, 132.3, 128.0, 127.4. 127.3, 126.6, 125.6, 124.9, 123.9, 123.6, 122.6, 122.4, 115.7, 114.9, 113.9, 106.3, 41.8, 41.3, 19.6, 13.2; anal. calcd for $C_{35}H_{30}Br_2N_4O_2S$ (%): C, 57.55; H, 4.14; N, 7.67; S, 4.39; found: C, 57.76; H, 4.18; N, 7.58; S, 4.32.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7c. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6c** (0.69 g, 3.92 mmol) and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) yielded the target hybrid phenothiazinyldihydropyridinedicarboxamide **7c**. Yield, 1.02 g (91%); MP: 152–153 °C; FT-IR (KBr, cm^{-1}): ν 3647.3, 3554.8, 3290.5, 2966.5, 2877.7, 2358.9, 1797.6, 1597.0, 1508.3, 1438.2, 1436.9, 1398.3, 1330.8, 1224.8, 1193.9, 1159.2, 1018.4, 941.2, 842.8, 748.3, 686.6, 607.5, 528.5, 430.1; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 2H), 8.18–6.82 (m, 17H), 5.90 (s, 1H), 4.76 (s, 1H), 3.89 (d, J = 6.80 Hz, 2H), 2.24 (s, 6H), 1.38 (t, J = 7.20 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.2, 148.3, 145.0, 144.3, 140.7, 139.3, 139.2, 129.6, 127.5, 127.4, 126.3, 125.6, 125.4, 123.3, 122.6, 118.4, 115.4, 115.3, 114.4, 105.7, 41.9, 40.9, 18.6, 12.9; anal. calcd for $C_{35}H_{32}N_4O_2S$ (%): C, 73.40; H, 5.63; N, 9.78; S, 5.60; found: C, 73.57; H, 5.56; N, 9.67; S, 5.66.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7d. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6d** (0.77 g, 3.92 mmol) and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) provided the target hybrid phenothiazinyldihydropyridinedicarboxamide **7d**. Yield, 0.97 g (82%); MP: 224–226 °C; FT-IR (KBr, cm^{-1}): ν 3425.5, 3037.8, 2954.9, 2829.5, 2669.4, 2370.5, 1917.2, 1805.3, 1622.1, 1593.2, 1498.6, 1450.4, 1398.3, 1346.3, 1224.3, 1165.0, 1124.5, 1033.8, 1006.8, 952.8, 833.2, 752.8, 833.2, 750.3, 684.7, 615.2, 578.6, 424.3; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 2H), 7.70–6.92 (m, 15H), 5.89 (s, 1H), 4.68 (s, 1H), 3.85 (br.d, J = 6.00 Hz, 2H), 2.30 (s, 6H), 1.39 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.2, 148.6, 145.5, 144.4, 140.3, 139.6, 139.4, 129.3, 127.7, 127.4, 126.3, 125.6, 125.4, 123.3, 122.7, 118.5, 115.3, 115.2, 114.4, 105.7, 41.8, 40.8, 18.7, 13.0; anal. calcd for $C_{35}H_{30}F_2N_4O_2S$ (%): C, 69.06; H, 4.97; N, 9.20; S, 5.27; found: C, 69.22; H, 4.90; N, 9.32; S, 5.21.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7e. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6e** (0.80 g, 3.92 mmol) and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) gave the target hybrid phenothiazinyldihydropyridinedicarboxamide **7e**. Yield, 84%; MP: 182–184 °C; FT-IR (KBr, cm^{-1}): ν 3664.7, 3307.9, 2966.5, 2881.6, 1940.3, 1884.4, 1797.6, 1618.2, 1593.2, 1512.1, 1483.2, 1450.4, 1400.3, 1377.1, 1232.5, 1166.9, 1122.5, 1076.2, 1028.0, 999.1, 950.9, 827.4, 752.2, 723.3, 663.5, 534.2, 435.9; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (s, 2H), 7.46–6.68 (m, 15H), 3.86 (d, J = 5.60 Hz, 2H), 2.64 (s, 6H), 2.52 (s, 6H), 2.43 (s, 6H), 1.23 (t, J = 7.10 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.6, 147.9, 145.4, 144.3, 143.8, 142.4, 129.5, 128.5, 127.7, 126.7, 124.8, 122.4, 122.3, 121.5, 114.0, 113.8, 112.4, 110.0, 104.7, 40.3, 22.3, 21.6, 20.9, 12.4; anal. calcd for $C_{39}H_{40}N_4O_2S$ (%): C, 74.49; H, 6.41; N, 8.91; S, 5.10; found: C, 74.70; H, 6.34; N, 8.86; S, 5.17.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7f. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6f** (1.0 g, 3.92 mmol) and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) provided the target hybrid phenothiazinyldihydropyridinedicarboxamide **7f**. Yield, 1.25 g (87%); MP: 206–208 °C; FT-IR (KBr, cm^{-1}): ν 3884.6, 3782.4, 3630.0, 3547.0, 3236.5, 3039.8, 2966.5, 2872.0, 2360.8, 1716.6, 1666.5, 1593.2, 1508.3, 1442.7, 1381.0, 1232.5, 1188.1, 1120.6, 1045.4, 952.8, 889.1, 827.4, 746.4, 725.2, 686.6, 613.3, 528.5, 432.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (2s, 2H), 7.58–6.77 (m, 15H), 5.52 (s, 1H), 4.83 (s, 1H), 3.87 (q, J = 6.80 Hz, 2H), 2.31 (s, 6H), 1.37 (t, J = 6.80 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.6, 144.8, 144.5, 139.9, 138.7, 136.1, 132.3, 132.2, 128.1, 127.3, 126.1, 125.5, 124.8, 123.9, 115.7, 114.9, 113.8, 106.4, 41.8, 41.4, 19.1, 13.0; anal. calcd for $C_{35}H_{30}Br_2N_4O_2S$ (%): C, 57.55; H, 4.14; N, 7.67; S, 4.39; found: C, 57.74; H, 4.08; N, 7.60; S, 4.45.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7g. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6g** (0.87 g, 3.92 mmol) and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) yielded the target hybrid phenothiazinyldihydropyridinedicarboxamide **7g**. Yield, 1.14 g (88%); MP: 160–162 °C; FT-IR (KBr, cm^{-1}): ν 3664.7, 3437.1, 3055.2, 2754.3, 1595.1, 1537.2, 1496.7, 1448.5, 1408.0, 1382.9, 1355.9, 1328.9, 1290.3, 1215.3, 1215.1, 1155.3, 1072.4, 1018.4, 964.4, 941.2, 817.8, 756.1, 731.0, 688.5, 516.9, 466.7; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.92 (s, 2H), 8.75 (s, 2H), 8.45 (s, 2H), 8.03–7.89 (m, 4H), 7.63–7.54 (m, 2H), 7.28–6.87 (m, 6H), 5.13 (s, 1H), 3.87 (br.d, J = 6.40 Hz, 2H), 2.18 (s, 6H), 1.27 (t, J = 6.20 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.4, 144.8, 143.1, 141.6, 141.1, 139.3, 130.4, 128.0, 127.4, 126.7, 126.2, 125.9, 123.1, 123.0, 122.6, 117.7, 115.8, 115.5, 113.9, 105.9, 41.4, 40.9, 18.0, 13.1; anal. calcd for $C_{35}H_{30}N_6O_6S$ (%): C, 63.43; H, 4.56; N, 12.68; S, 4.84; found: C, 63.65; H, 4.48; N, 12.52; S, 4.91.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7h. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6h** (0.93 g, 3.92 mmol) and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) furnished the target hybrid phenothiazinyldihydropyridinedicarboxamide **7h**. Yield, 1.19 g (92%); MP: 242–244 °C; FT-IR (KBr, cm^{-1}): ν 3238.5, 3140.1, 3076, 2833.4, 1637.6, 1606.7, 1514.1, 1465.9, 1408.0, 1328.9,

1257.6, 1238.3, 1138.0, 1028.1, 964.4, 923.9, 846.8, 800.5, 748.4, 613.4, 565.1; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90 (s, 2H), 7.49–6.66 (m, 15H), 3.85 (br.s, 8H), 3.63 (s, 6H), 2.45 (s, 6H), 1.23 (t, J = 6.40 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.3, 154.6, 147.8, 145.4, 144.2, 142.9, 129.6, 128.5, 127.7, 126.7, 126.4, 126.2, 125.8, 123.4, 122.3, 121.6, 114.0, 113.9, 112.4, 109.9, 104.6, 55.0, 54.6, 40.9, 21.6, 11.6; anal. calcd for $C_{39}H_{40}N_4O_6S$ (%): C, 67.61; H, 5.82; N, 8.09; S, 4.63; found: C, 67.84; H, 5.90; N, 8.00; S, 4.57.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7i. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6i** (0.77 g, 3.92 mmol) and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) gave the target hybrid phenothiazinyldihydropyridinedicarboxamide **7i**. Yield, 0.96 g (80%); MP: 208–210 °C; FT-IR (KBr, cm^{-1}): ν 3880.7, 3817.1, 3628.1, 3039.8, 2999.3, 2902.8, 2831.5, 2673.3, 2370.5, 1809.2, 1664.5, 1602.8, 1506.4, 1454.3, 1398.3, 1350.1, 1296.1, 1244.0, 1172.7, 1031.9, 954.7, 835.1, 750.3, 615.2, 513.0, 435.9; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (d, 2H), 7.65 (s, 2H), 7.52 (s, 2H), 7.35–6.73 (m, 11H), 5.67 (s, 1H), 4.83 (s, 1H), 3.87 (br.d, J = 6.80 Hz, 2H), 2.33 (s, 6H), 1.35 (t, J = 7.00 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.6, 144.8, 144.2, 140.0, 138.7, 136.1, 134.9, 132.4, 132.2, 128.4, 127.1, 126.6, 125.6, 124.8, 124.0, 122.6, 115.7, 115.0, 114.6, 113.8, 106.3, 41.8, 41.4, 19.1, 12.6; anal. calcd for $C_{35}H_{30}F_2N_4O_2S$ (%): C, 69.06; H, 4.97; N, 9.20; S, 5.27; found: C, 69.31; H, 4.88; N, 9.11; S, 5.35.

Hybrid phenothiazinyldihydropyridinedicarboxamide 7j. A mixture of carbaldehyde **3** (0.5 g, 1.96 mmol), aryloxobutanamide **6j** (0.81 g, 3.92 mmol) and ammonium acetate (0.23 g, 2.94 mmol) in the presence of *p*-toluenesulfonic acid (15 mol%) in ethanol (15 mL) by adopting the general method followed by column chromatography (30–50% ethyl acetate in hexane) gave the target hybrid phenothiazinyldihydropyridinedicarboxamide **7j**. Yield, 1.03 g (84%); MP: 232–234 °C; FT-IR (KBr, cm^{-1}): ν 3547.0, 3315, 3283, 3047.5, 2972.3, 2875.8, 1942.3, 1593.2, 1512.1, 1392.6, 1338.6, 1188.1, 1111.0, 1080.1, 1045.4, 966.3, 889.1, 858.3, 754.1, 721.3, 686. 6, 619.1, 536.2, 499.5, 432.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.91 (s, 2H), 7.33–6.56 (m, 15H), 3.86 (br.d, J = 5.60 Hz, 2H), 2.62 (s, 6H), 2.53 (s, 6H), 2.42 (s, 6H), 2.30 (s, 6H), 2.23 (s, 6H), 1.22 (t, J = 7.08 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.4, 154.6, 147.9, 145.3, 144.3, 142.8, 142.4, 129.5, 128.5, 127.7, 126.7, 126.4, 126.2, 125.8, 123.4, 122.2, 121.5, 114.0, 113.9, 112.4, 109.9, 104.7, 41.6, 22.2, 22.0, 21.3, 11.5; anal. calcd for $C_{39}H_{40}N_4O_2S$ (%): C, 74.49; H, 6.41; N, 8.91; S, 5.10; found: C, 74.78; H, 6.50; N, 8.82; S, 5.05.

Anti-inflammatory activity by the protein denaturation method

The methods of Mazushima *et al.*[42] and Sakat *et al.*[43] were used with a slight modification for the *in vitro* anti-inflammatory study. The reaction mixture (2.5 mL) consisted of synthetic derivatives (1 mL; 1 mM), egg albumin (0.1 mL) and phosphate buffered saline (PBS, 1.4 mL; pH 6.4). Double distilled water was utilized as the control (equal volume). The mixture was then heated at 70 °C for 5 min after incubation at 37 °C ± 2 for 15 min. Their absorbance, after cooling, was measured at 660 nm utilizing the vehicle as a blank. As a reference drug, diclofenac sodium (1 mM) was utilized and treated similarly for determination of the absorbance. The inhibition (%) of protein denaturation was then calculated.

Radical scavenging activity by the DPPH method

The radical scavenging assay (DPPH) was carried out according to the literature[44] with minor modifications. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (1.6 mg) was dissolved in DMSO (50 mL). To 1.5 mL of each compound prepared (100 μg mL^{-1}) was added 1.5 mL of DPPH solution and the mixture was then kept for 45 min incubation under dark conditions at room temperature. The changes in absorbance at 517 nm were then measured. The absorbance at 517 nm of the blank DPPH solution was used as the control. The DPPH free radical scavenging activity was then calculated.

Anticancer evaluation

Human pancreatic cancer cells SW1990, AsPC1, BxPC3 and Panc1 as well as non-cancerous lung fibroblast MRC5 were procured from the American Type Culture Collection, ATCC, USA. All the cells were cultured in RPMI-FBS medium supplemented with 100 IU mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin (Sigma-Aldrich, St. Louis, MO, USA). All the pancreatic cancer cells were incubated at 37 °C in 5% CO_2, using the standard *in vitro* cell culture method established previously.[47–51]

In order to evaluate the antitumor effects of the synthesized chemical entities, stock solutions of the synthesized compounds in DMSO (100 mM) were initially prepared, then further diluted to 0.1 mM in sterile PBS. The cells were treated based on the cell viability assay reported in the literature.[47,52] First, 384-well plates were seeded with cells in such a way that each well contained 1500 cells per well. Plates were incubated for 24 h and then the cells were treated with the synthesized compounds for 72 h. Cell viability was measured using the Cell-Titre Glo Luminescent Cell Viability Assay (Promega, USA). The luminescence readings were recorded using a SpectraMax M3 microplate reader (Molecular Devices Corporation, USA).

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

Financial assistance provided by the Indian Council of Medical Research, New Delhi, India (No. 58/16/2013BMS) is gratefully acknowledged.

References

1 J. E. Niederhuber, M. F. Brennan and H. R. Menck, *Cancer*, 1995, **76**, 1671–1677.
2 Z. Wang, A. Ahmad, Y. Li, A. Azmi, L. Miele and F. Sarkar, *Anticancer Res.*, 2011, **31**, 1105–1113.

3 H. G. O. Alvim, E. N. da Silva Júnior and B. A. D. Neto, *RSC Adv.*, 2014, **4**, 54282–54299.
4 R. C. Cioc, E. Ruijter and R. V. A. Orru, *Green Chem.*, 2014, **16**, 2958–2975.
5 (*a*) D. J. Ramón and Y. Miguel, *Angew. Chem., Int. Ed.*, 2005, **44**, 1602–1634; (*b*) R. V. A. Orru and M. De Greef, *Synthesis*, 2003, 1471–1499; (*c*) I. Ugi and S. Heck, *Comb. Chem. High Throughput Screening*, 2001, **4**, 1–34; (*d*) L. Weber, K. Illgen and M. Almstetter, *Synlett*, 1999, 366–374.
6 (*a*) B. E. Evans, K. E. Rittle, M. G. Bock, R. M. DiPardo, R. M. Freidinger, W. L. Whitter, G. F. Lundell, D. F. Veber, P. S. Anderson, R. S. L. Chang, V. J. Lotti, D. J. Cerino, T. B. Chen, P. J. Kling, K. A. Kunkel, J. P. Springer and J. Hirshfield, *J. Med. Chem.*, 1988, **31**, 2235–2246; (*b*) A. A. Patchett and R. P. Nargund, *Annu. Rep. Med. Chem.*, 2000, **35**, 289–298.
7 (*a*) N. Edraki, A. R. Mehdipour, M. Khoshneviszadeh and R. Miri, *Drug Discovery Today*, 2009, **14**, 1058–1066; (*b*) K. Sirisha, D. Bikshapathi, G. Achaiah and V. M. Reddy, *Eur. J. Med. Chem.*, 2011, **46**, 1564–1571.
8 U. Eisner and J. Kuthan, *Chem. Rev.*, 1972, **72**, 1–42.
9 (*a*) D. Schade, M. Lanier, E. Willems, K. Okolotowicz, P. Bushway, C. Wahlquist, C. Gilley, M. Mercola and J. R. Cashman, *J. Med. Chem.*, 2012, **55**, 9946–9957; (*b*) A. Mai, S. Valente, S. Meade, V. Carafa, M. Tardugno, A. Nebbioso, A. Galmozzi, N. Mitro, E. D. Fabiani, L. Altucci and A. Kazantsev, *J. Med. Chem.*, 2009, **52**, 5496–5504; (*c*) F. Lentz, M. Hemmer, N. Reiling and A. Hilgeroth, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 5896–5898.
10 A. Berntsen, *Ber. Dtsch. Chem. Ges.*, 1883, **16**, 2896–2904.
11 R. R. Gupta and M. Kumar, Synthesis, properties and reactions of phenothiazines, in *Phenothiazines and 1,4-Benzothiazines – Chemical and Biological Aspects*, ed. R. R. Gupta, Elsevier, New York, 1988, pp. 1–161.
12 I. A. Silberg, G. Cormos and D. C. Oniciu, Retrosynthetic approach to the synthesis of phenothiazines, in *Advances in heterocyclic chemistry*, Elsevier, New York, 2006, vol. 90, pp. 205–237.
13 ECM, Phenothiazine, European Chemicals Bureau IUCLID Dataset, 2000.
14 R. R. Reddyrajula, U. Dalimba and S. Madan Kumar, *Eur. J. Med. Chem.*, 2019, **15**, 263–282.
15 K. Sroda-Pomianek, K. Michalak, A. Palko-Labuz, A. Pola, P. Dziegiel, B. Pula, P. Swiatek and O. Wesolowska, *Eur. J. Pharmacol.*, 2019, **849**, 124–134.
16 M. M. Ghorab, A. S. Alsaid, N. Samir, G. A. Abdel-Latif, A. M. Soliman, F. A. Ragab and D. A. Abou El Ella, *Eur. J. Med. Chem.*, 2017, **134**, 304–315.
17 B. Varga, A. Csonka, A. Csonka, J. Molnar, L. Amaral and G. Spengler, *Anticancer Res.*, 2017, **37**, 5983–5993.
18 J. E. Kristiansen, S. G. Dastidar, S. Palchoudhuri, D. Sinha Roy, S. Das, O. Hendricks and J. B. Christensen, *Int. Microbiol.*, 2015, **18**, 1–12.
19 P. Songarj, C. Luh, I. Staib-Lasarzik, K. Engelhard, B. Moosmann and S. C. Thal, *Neurosci. Lett.*, 2015, **584**, 253–258.
20 A. N. Matralis and A. P. Kourounakis, *J. Med. Chem.*, 2014, **57**, 2568–2581.
21 A. Jaszczyszyn, K. G. Asiorowski, P. Swiatek, W. Malinka, K. Cieslik-Boczula, J. Petrus and B. Matusewicz-Czarnik, *Pharmacol. Rep.*, 2012, **64**, 16–23.
22 O. Wesolowska, *Acta Biochim. Pol.*, 2011, **58**, 433–448.
23 G. Sudeshna and K. Parimal, *Eur. J. Pharmacol.*, 2010, **648**, 6–14.
24 G. C. González-Muñoz, M. P. Arce, B. López, C. Pérez, A. Romero, L. del Barrio, M. D. Martín-de-Saavedra, J. Egea, R. León, M. Villarroya, M. G. López, A. G. García, S. Conde and M. I. Rodríguez-Franco, *Eur. J. Med. Chem.*, 2010, **45**, 6152–6158.
25 J. J. Aaron, M. D. Gaye Seye, S. Trajkovska and N. Motohashi, *Top. Heterocycl. Chem.*, Springer-Verlag, Berlin, 2009, vol. 16, pp. 153–231.
26 K. Kubota, H. Kurebayashi, H. Miyachi, M. Tobe, M. Onishi and Y. Isobe, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 2766–2771.
27 A. Bisi, M. Meli, S. Gobbi, A. Rampa, M. Tolomeo and L. Dusonchet, *Bioorg. Med. Chem.*, 2008, **16**, 6474–6482.
28 A. Dasgupta, S. G. Dastridara, Y. Shirataki and N. Motohashi, *Top. Heterocycl. Chem.*, Springer Verlag, Berlin, 2008, vol. 15, pp. 67–132.
29 A. D. Mosnaim, V. V. Ranade, M. E. Wolf, J. Puente and M. A. Valenzuela, *Am. J. Therapeut.*, 2006, **13**, 261–273.
30 N. Motohashi, M. Kawase, K. Satoh and H. Sakagami, *Curr. Drug Targets*, 2006, **7**, 1055–1066.
31 A. Rajasekaran and P. P. Thampi, *Eur. J. Med. Chem.*, 2004, **39**, 273–279.
32 Y.-H. Jiao, F.-Y. Meng, G.-B. Zhu, L.-Z. Ran, Y.-F. Jiang and Q. Zhang, *Exp. Ther. Med.*, 2019, **17**, 1321–1329.
33 B. Mathew, T. S. Snowden, M. C. Connelly, R. K. Guy and R. C. Reynolds, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 2136–2142.
34 S. Hassan, S. A. Ejaz, A. Saeed, M. Shehzad, S. U. Khan, J. Lecka, J. Sévigny, G. Shabir and J. Iqbal, *Bioorg. Chem.*, 2018, **76**, 237–248.
35 G. Verma, G. Chashoo, A. Ali, M. F. Khan, W. Akhtar, I. Ali, M. Akhtar, M. M. Alam and M. Shaquiquzzaman, *Bioorg. Chem.*, 2018, **77**, 106–124.
36 K. Padmavathy, K. Gokula Krishnan, C. Udhaya Kumar, P. Sutha, R. Sivaramakarthikeyan and C. Ramalingan, *ChemistrySelect*, 2018, **3**, 5965–5974.
37 K. Gokula Krishnan, P. Ashothai, K. Padmavathy, W.-M. Lim, C.-W. Mai, V. T. Punniyakoti and C. Ramalingan, *New J. Chem.*, 2019, **43**, 12069–12077.
38 K. Padmavathy, K. Gokula Krishnan, C. Udhaya Kumar, E. Sathiyaraj, R. Sivaramakarthikeyan, W.-M. Lim, C.-W. Mai and C. Ramalingan, *New J. Chem.*, 2019, **43**, 13418–13429.
39 K. Gokula Krishnan, C. Udhaya Kumar, W.-M. Lim, C.-W. Mai, V. T. Punniyakoti and C. Ramalingan, *J. Mol. Struct.*, 2020, 1199, DOI: 10.1016/j.molstruc.2019.127037.
40 K. Stalindurai, A. Karuppasamy, J. D. Peng, K.-C. Ho and C. Ramalingan, *Electrochim. Acta*, 2017, **246**, 1052–1064.
41 W. Liu, P. Zhou, C. Chen, Q. Zhang and Z. Zhu, *Org. Biomol. Chem.*, 2013, **11**, 542–544 and references cited therein.
42 Y. Mizushima and M. Kobayashi, *J. Pharm. Pharmacol.*, 1968, **20**, 169–173.
43 S. Sakat, A. R. Juvekar and M. N. Gambhire, *Int. J. Pharm. Pharm. Sci.*, 2010, **2**, 146–155.

44 S. E. Atawodi, *Afr. J. Biotechnol.*, 2004, **4**, 128–133.
45 J. M. Braughler, L. A. Duncan and R. L. Chase, *J. Biol. Chem.*, 1986, **261**, 10282–10289.
46 M. P. Rajesh and J. P. Natvar, *J. Adv. Pharm. Educ. Res.*, 2011, **1**, 52–68.
47 J. L. Er, P. N. Goh, C. Y. Lee, Y. J. Tan, L.-W. Hii, C. W. Mai, F. F.-L. Chung and C.-O. Leong, *Apoptosis*, 2018, **23**, 343–355.
48 F. F.-L. Chung, P. F. T. M. Tan, V. J. Raja, B.-S. Tan, K.-H. Lim, T.-S. Kam, L.-W. Hii, S. H. Tan, S.-J. See, Y.-F. Tan, L.-Z. Wong, W. K. Yam, C. W. Mai, T. D. Bradshaw and C.-O. Leong, *Sci. Rep.*, 2017, **7**, 42504.
49 K. Y. Yeong, S. C. Tan, C.-W. Mai, C.-O. Leong, F. F.-L. Chung, Y. K. Lee, C. F. Chee and N. A. Rahman, *Chem. Biol. Drug Des.*, 2018, **91**, 213–219.
50 C.-W. Mai, Y.-B. Kang, V. D. Nadarajah, A. S. Hamzah and M. R. Pichika, *Phyther. Res.*, 2018, **32**, 1108–1118.
51 C. W. Mai, K. S. I. Yap, M. T. Kho, N. H. Ismail, K. Yusoff, K. Shaari, S. Y. Chin and E. S. H. Lim, *Front. Pharmacol.*, 2016, **7**, 1–11.
52 H.-C. Soo, F. F.-L. Chung, K.-H. Lim, V. A. Yap, T. D. Bradshaw, L.-W. Hii, S.-H. Tan, S.-J. See, Y.-F. Tan, C.-O. Leong and C.-W. Mai, *PLoS One*, 2017, **12**, 1–20.