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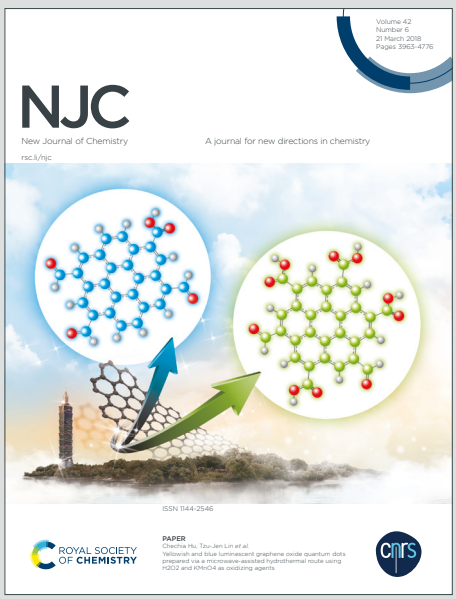

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Phenothiazine and amide ornamented novel nitrogen heterocyclic hybrids: Synthesis and their biological and molecular docking studies

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Synthesis of novel hybrids, phenothiaze and amide ornamented nitrogen heterocycles 25-34 has been accomplished utilizing multi-step synthetic protocol and the structures have been established based on physical and spectral techniques. Of those, the hybrids possessing meta- nitro (26), para- fluoro (28), meta- and para- methyl (31), ortho- bromo (33) and ortho- and para- dimethyl (34) phenyl carboxamide scaffold exhibited superior anti-inflammatory profile over the standard diclofenac sodium. A hybrid integrated with para- fluorophenyl carboxamide moiety (28) showed the highest DPPH radical scavenging activity among the chemical entities synthesized. Further, the results of anticancer evaluation implied that the hybrid tethered with phenyl carboxamide structural unit (29) exerted superior activity when compared with other hybrids against the pancreatic cancer cells SW1990 and AsPCl. Molecular docking between the hybrid 29 and B-cell lymphoma 2 reflects its appreciable binding affinity (-8.84 Kcal / mol). The results revealed that these chemical entities could serve as potent biological agents and / or serve as efficient intermediates for the construction of potent biological agents.

Introduction

One of the foremost threats to universal health is non-communicable diseases (NCDs) that are the origin for significant number of deaths each year. Cancer is the second most important cause of death in the world and caused by abnormalities in cells, might be either because of exposure to radiation / chemicals / even infectious agents or inherited. The speedy propagation of unusual cells that develop away from the usual boundaries, and then attacks adjacent parts of the body and extending to other organs is one of the typical characters of cancer. Stomach, lungs, pancreas, liver, breast and colon are the usually affected organs, which culminated with death every year. This non-communicable disease is a main health defy not only in developed nations but also in developing and under developed nations, where the number of occurrences of the same is rapidly growing.[1] There are 9.6 million deaths around the globe in 2018 due to this life-threatening disease and one in six deaths is because of the same. It is expected that the world death toll from cancer is continuing to increase (~12 million deaths by 2030).[2]

There are several types of treatments for cancer available and the type of treatment depends on the type of cancer as well as its advancement. Broad types of cancer treatment comprise chemotherapy, surgery, immunotherapy, radiation therapy, targeted therapy, hormone therapy and stem cell transplant etc. Cancer and cancer treatment can cause one or more side effects including appetite loss, constipation, fertility issues in women and men, hair loss, memory or concentration problems, nerve problems, pain, sexual health issues in women and men, skin and nail changes, urinary and bladder problems etc. There are hundreds of drugs commercially available in the market to treat cancers, regrettably, most of them are allied with severe side effects and consequently, it is a challenging task to save lives. The rising rate of death year by year, as a reason of cancer, has made apparent that there is an abrupt need for the development of anticancer agents with more potential that can eradicate cancer cells without harming regular tissues. In this track, diverse design approaches are being confirmed to identify potent molecules.

Molecular hybridization is an influential tool for designing a drug that engrosses the hybridization of two or more potent pharmacophores of either dissimilar biopertinent chemical entities or similar biopertinent chemical entities into one molecular scaffold.[3] It is being extensively used due to the fact that numerous molecules of such crossbreed nature exhibited enhanced efficiency besides safer toxicity report, while comparing with their parent molecules. Based on this strategy, several biologically potent structural motifs, mainly heterocyclic units are being investigated to recognize novel lead compounds.

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Dihydropyrimidines, a class of molecules possessing a six membered cyclic structure with nitrogens at 1 and 3 positions, are an imperative category of heterocycles. Over the few decades, the interest on the same among the researchers has been increased because of a couple of independent reasons. One is comparatively easiness of the construction of the ring structure with wide variety of derivatization[4] and the other is their recognition as valuable pharmacophores exhibiting a wide variety of pharmacological profile including anti-inflammatory,[5] adrenergic antagonists,[6] antihypertension,[7] antimicrobial[8] and so on. Of the research on biological aspects, the interest in the anticancer properties of pyrimidines has increased rapidly since the discovery of Monastrol, a novel low molecular weight, cell permeable chemical entity that also cause mitotic arrest in mammalian cells in mitosis with monopolar spindles, in 1999.[9] Representative examples of biopertinent chemical entities possessing dihydropyrimidine scaffold are furnished in **Figure 1**.

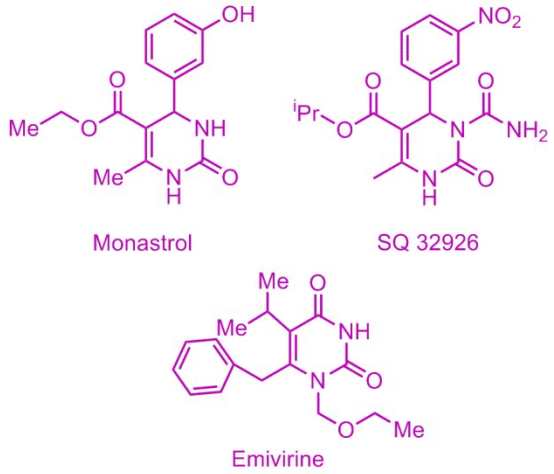


Fig 1. Representative examples of dihydropyrimidines with biological importance

Fused thiazines, especially phenothiazines are documented since nineteenth century when Bernthsen synthesized the first parent molecule, 10H-dibenzo-1,4-thiazine in 1883.[10] Because of their striking chemical character besides noteworthy pharmacological / biological potency, their construction, plausible reactions, properties, and applications especially pharmacological / biological angle were scrupulously reviewed three decades ago and retrosynthetic approach for the construction of phenothiazine was also depicted.[11,12]

In the recent years, numerous articles related to these fused thiazines were appeared and until present, over 6000 derivatives of the same were attained. Further, a minimum ten dozen of these derivatives were exploited in treatment. An important aspect of this scaffold is its capability to offer a wide range of analogues (substituents could be incorporated at various positions besides oxidation of sulphur and replacement of homoaryl by heteroaryl units viz., pyrimidine, pyridine, quinoline, pyridazine, 1,2,4-triazine, and pyrazine and so on). In addition, this fused thiazine is reported to be harmless, an unusual one, and it is tolerable up to doses of multi-gram scale in humans.[13] A diverse range of molecules incorporated with phenothiazine unit exhibit various pharmacological/biological properties. Their striking characteristics were reviewed recently.[14-31]

With respect to anticancer aspects, examination on chlorpromazine **(Figure 2)**, a phenothiazine pharmacore possessing drug, revealed that it can augment effects of cytotoxicity of tamoxifen on the cancer associated with breast.[32] Molecules possessing phenothiazine structural motif can also improve sensitivity of cisplatin, a drug being used for chemotherapy.[33] Particularly, a couple of derivatives of phenothiazine such as (2,4-dimethoxyphenyl)(10H-phenothiazin-10-yl)methanone have been examined in order to understand their ability to hamper antiproliferative activity and tubulin polymerization against six dozens of cancer cell lines.[34] The chemical entities exhibited notable inhibition of cell growth on COLO 205 (colorectal adenocarcinoma), A498 (human kidney adenocarcinoma) and Duke's type D (human colon) cell lines.[34] All these noteworthy results recommended that phenothiazine scaffold could be considered as a capable anticancer pharmacophore in drug discovery.

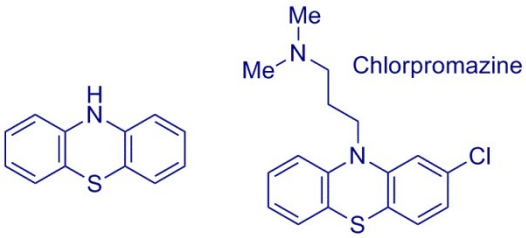


Fig 2. Representative anticancer molecules possessing phenothiazine structural motif

On the other hand, amide moiety is a vital structural motif which serves as possible ligand part in the interaction of a chemical entity with host proteins through H- bonding and / or vander-Waals power of attractions. Chemical entities integrated with amide scaffolds exhibit diverse biological activities that include antimalarial and anticancer etc.[35-38]

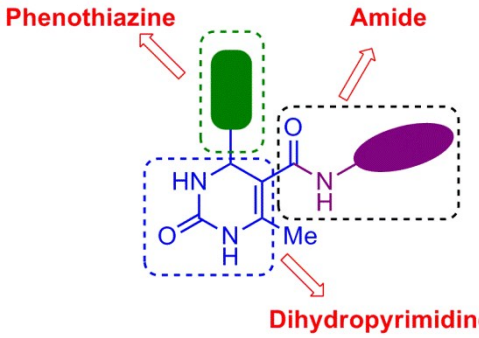


Fig 3. Dihydropyridine, phenothiazine and amide hybridized novel chemical entities

Having the biological magnitude of the aforementioned scaffolds in mind, a series of fused thiazine, dihydropyrimidine and amide hybridized novel molecules has been designed and synthesized as biopertinent ones. Since it is known that cancer is a type of inflammation, and also it is known that the ROC may influence the cellular components which cause cancer, preliminary anti-inflammatory evaluation and plausible radical

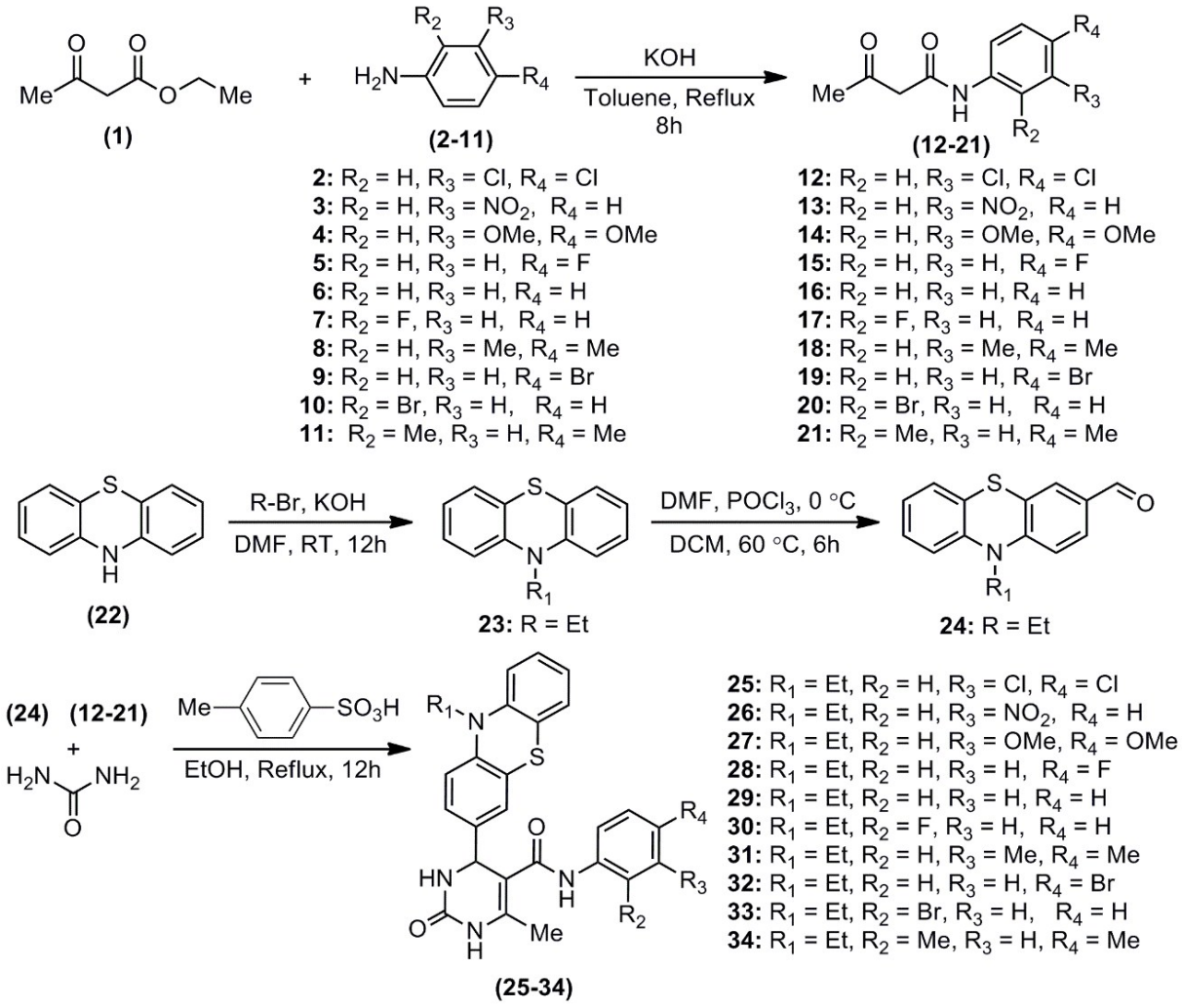
scavenging activities of the novel molecules have been determined. Further, anticancer evaluation of all the novel chemical entities have been executed against pancreatic cancer cells (human) SW1990 and AsPCl. Described herein are synthesis, characterization, anti-inflammatory, radical scavenging and anticancer evaluations as well as molecular docking studies of novel hybrids, phenothiazenyldihydropyrimidine carboxamides as provided in **Figure 3**.

Results and discussion

Chemistry

The sketch of the synthetic scheme for the construction of hybrid phenothiazinyltetrahydropyrimidinecarboxamides **25-34** is furnished in **Scheme 1**. Firstly, the synthesis of intermediates, 3-oxo-N-substituted phenylbutanamides **12-21** has been achieved from the reaction of β-keto ester **1** with appropriately substituted anilines **2-11** in the presence of KOH in toluene.[39] On the other hand, synthesis of the active intermediate phenothiazine carbaldehyde **24** has been accomplished from commercial phenothiazine **22** by adopting alkylation utilising bromoalkane in the presence of KOH in DMF followed by Vilsmeier-Haack reaction with the utilization of $POCl_3$ and DMF in dichloromethane.[40] A multicomponent one-step reaction between appropriate 3-oxo-N-substituted phenylbutanamides **12-21**, phenothiazine carbaldehyde **24**, and urea in the ratio of 1.1:1:1.5, respectively in the presence of p-toluenesulfonic acid in ethanol eventually produced the desired target hybrid phenothiazinyltetrahydropyrimidine carboxamides **25-34** in good yields. The structures of novel phenothiazinyltetrahydropyrimidinecarboxamides have unequivocally been established based on physical and spectral methods.

In the proton NMR spectra of the target phenothiazinyltetrahydropyrimidine carboxamides **25-34**, one of the secondary amino protons of dihydropyrimidine structural unit resonates between 5.40 and 6.38 ppm as a singlet with one proton integral while signal for the other secondary amino proton of dihydropyrimidine scaffold merged with aromatic signals. The proton connected to the nitrogen of the amide moiety resonate from 7.34 to 8.15 ppm as a singlet with one proton integral except in **27-29**, wherein the signal for this proton is merged with aromatic ones. The protons of



Scheme 1. Synthesis of novel hybrid phenothiazinyltetrahydropyrimidine carboxamides **25-34**

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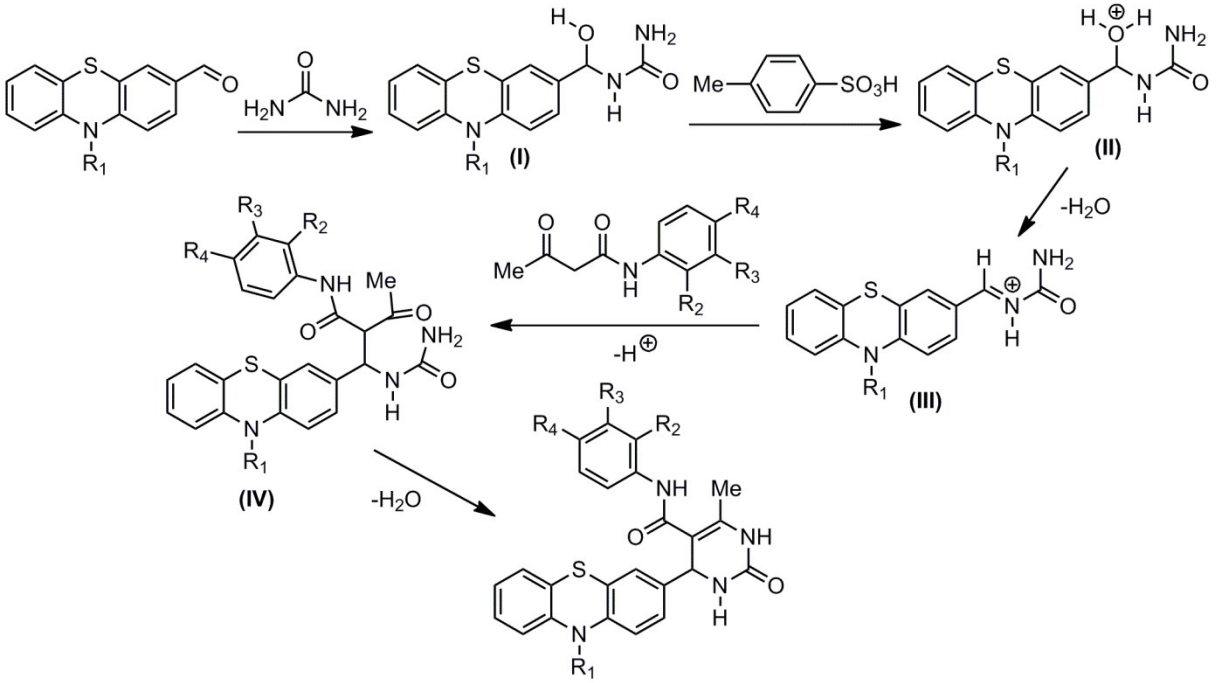
the methyl group attached to the 6-position of dihydropyrimidine core offer a singlet with three protons integral in the region 2.15 to 2.28 ppm while the protons of the methyl moiety of ethyl unit attached to phenothiazine observed in the region 1.28 to 1.39 ppm as a three-proton triplet. The methylene protons of the ethyl moiety tethered at nitrogen of the phenothiazine resonate in the region from 3.78 to 3.89 ppm as either broad doublet or quartet with two protons integral. In addition, the methine proton connected at 4-position of dihydropyrimidine resonates in the region between 5.16 and 5.27 ppm as a one proton singlet while the aromatic protons of the targets exert multiplet in the region between 6.59 and 7.93 ppm. Besides, the protons of methyl groups tethered at aromatic ring in **31** offer signals at 2.14 and 2.16 ppm as singlets with three protons integral while the ones attached to the aromatic scaffold in **34** provide signals at 2.17 and 2.23 ppm as singlets with three protons integral. Eventually, the protons of the methoxy units tethered to the aromatic scaffold in 27 resonate as three protons singlet at 3.76 and 3.81 ppm.

In the carbon NMR spectra of the target phenothiazinyltetrahydropyrimidine carboxamides **25-34**, the signal for the methyl carbon tethered at 6-positions of dihydropyrimidine scaffold resulted in the region from 16.82 to 18.00 ppm. The signals for methylene carbon (attached to nitrogen of phenothiazine unit) and methyl carbon of ethyl group observed in the region 40.64-41.91 ppm and 11.90-13.18 ppm, respectively. The methine carbon located at 4-position of dihydropyrimidine exert signal in the region from 54.63 to 55.89 ppm while the signal for carbon situated at 5-position of the dihydropyrimidine core observed in the region between 103.43 and 105.83 ppm. Further, the signal for carbon present at 6- position of the dihydropyrimidine resonate in the region between 144.42 and 145.66 ppm. The carbon of the carbonyl group of amide unit exhibits signal in the region from 163.36 to 165.75 ppm while the carbon of the carbonyl group located between two secondary amino moieties possesses signal between 152.63 and 153.46 ppm. In addition, the methyl carbons tethered at ortho- and para-positions of the phenyl ring in **34** offered signals at 20.80 and 17.94 ppm while the same attached at meta- and para-positions of the phenyl rings **31** provided signals at 20.04 and 19.22 ppm. Besides, the carbons of two methoxy moieties attached at meta- and para- positions of phenyl unit in **27** resonate at 56.28 and 56.11 ppm. Further, the signals for all the aromatic carbons observed in the region from 111.23 to 145.50 ppm. All these characteristics in addition to microanalysis and IR corroborate the target molecules.

The plausible mechanism for the formation of target molecules is furnished in **Scheme 2**. Initially, nucleophilic addition reaction between respective phenothiazine aldehyde and urea provides their corresponding hemiaminals, 1-(hydroxy(10-alkyl-10H-phenothiazin-3-yl)methyl)ureas **(I)**. The corresponding hemiaminals then abstracts proton from the p-toluenesulfonic acid to form respective oxonium ions, [(10-alkyl-10H-phenothiazin-3-yl)(ureido)methyl]oxonium ions **(II)** which subsequently loses water molecule to afford uronium ions, 1-[(10-alkyl-10H-phenothiazin-3-yl)methylene]uroniums **(III)**. Respective amides, synthesized previously from ethylacetoacetate and aromatic amines, eventually attacks the iminium carbon of the intermediates **III** to furnish open chain intermediates **IV** with elimination of proton which subsequently loses water to provide the title molecules.



Scheme 2. Plausible mechanism for the synthesis of target molecules **25-34.**

Biological activities

Anti-inflammatory activity

Anti-inflammatory evaluation (in vitro) of all the synthesized target phenothiazinyltetrahydropyrimidine carboxamides **25-34** has been carried out by employing the method of protein denaturation.[41,42] Protein denaturation is a technique in which the secondary and tertiary structure of protein would be lost when an external stress or a molecule is applied. The biological function of most biological proteins would be lost when denatured. As it is known, denaturation of proteins is a well-documented basis of inflammation. As a piece of the present exploration, capability of target molecules **25-34** for the inhibition of protein denaturation was assessed. The standard drug used in this investigation was diclofenac sodium which displayed ~90% inhibition of protein denaturation. The percentage inhibitions of protein denaturation upon the use of target molecules **25-34** are furnished with respect to the inhibition by standard diclofenac sodium and the results are provided in **Table 1** and **Figure 4**.

Table 1. Anti-inflammatory activity of **25-34**.

S.No.	Compound	% Inhibition
1	**25**	87.45 ± 0.99
2	**26**	100.67 ± 1.73
3	**27**	89.49 ± 2.14
4	**28**	100.68 ± 1.52
5	**29**	96.61 ± 2.41
6	**30**	97.63 ± 0.73
7	**31**	102.72 ± 0.69
8	**32**	75.25 ± 1.25
9	**33**	100.67 ± 1.45
10	**34**	104.74 ± 1.09

When the amide and phenothiazine integrated dihydropyrimidine having chloromoieties on the meta- and para- positions of the aryl unit of the amide group and ethyl moiety on the nitrogen of the phenothiazine **(25)** was used, it exhibits 87% inhibition on comparing to the inhibition of standard diclofenc sodium. Replacing the chloro moieties on the meta- position by nitro group and para- positions by hydrogen in **25** (i.e., **26**) enhanced the inhibition (13%) which is slightly higher than that of the standard drug. Incorporation of methoxy groups on the meta- and para- positions of the aryl ring in place of chloro moieties in **25** (i.e., in **27**) did not improve significantly the inhibition, it exhibits almost similar inhibition while the incorporation of bromo on the para- position of the aryl moiety instead of chloro unit and replacing the chloro group on the meta- position of the aryl group by hydrogen in **25** (i.e., in **32**) reduced the percentage of inhibition by 12%. The chemical entities with no substituents on the aryl scaffold of the amide unit **(29)** as well as fluoro substituent on the ortho- position of the aryl moiety of the amide unit **(30)** showed excellent results (97% and 98% inhibition, respectively) when compared to the standard drug. Further, integration of methyl substituents on the meta- and para- positions as well as ortho- and para- positions of the aryl group of amide unit (**31** and **34**, respectively) and also bromo substituent on the ortho- position of the aryl moiety of the amide unit **(33)** exhibited percentage inhibition superior than that of the standard though marginal improvement. Among all the molecules evaluated, **34** was found to be the most active one.

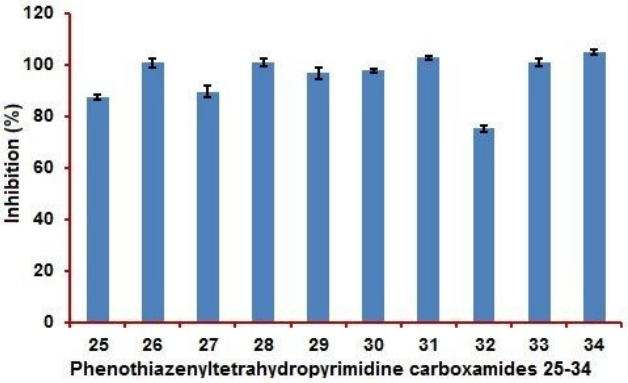


Fig 4. Antiinflammatory activity of **25-34** with respect to diclofenac sodium.

Radical scavenging activity

Reactive oxygen species (ROS) are basis for various neurodegenerative illnesses which include ulcer, Alzheimer's, mutagenesis, autoimmune disorder, and carcinogenesis by hampering the function of several cellular components. Antioxidants are agents that scavenge the free radicals formed in our biological system. Although a diverse range of such agents are present in nature, it is vital to develop a drug with superior antioxidant activity. For the determination of radical scavenging activity, various methodologies which include hydroxyl radical scavenging assay, ferric reducing antioxidant power, and organic radical scavenging assay are available. Among all of them, organic radical scavenging by 2,2-diphenyl-1-picrylhydrazyl (DPPH) is being extensively utilized by the researchers due to its simplicity. Accordingly, all the target phenothiazinyltetrahydropyrimidine carboxamides 25-34 have been examined for radical scavenging activity against DPPH, a reactive oxygen species.[43] The activity has been assessed in terms of inhibition of DPPH and the results are given in **Table 2** and **Fig. 5** on comparison with the activity of a standard ascorbic acid.

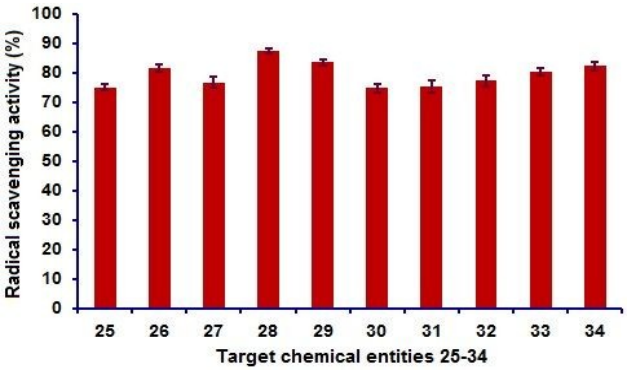


Fig 5. Radical scavenging activity of **25-34**

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Table 2. Radical scavenging of target molecules **25-34.**

S. No.	Molecule	% Scavenging	IC_{50} (μM)
1	**25**	75.03 ± 0.89	-
2	**26**	81.48 ± 1.28	47.63
3	**27**	76.71 ± 1.75	48.93
4	**28**	87.43 ± 0.80	103.80
5	**29**	83.48 ± 0.98	22.81
6	**30**	74.72 ± 1.38	61.63
7	**31**	75.23 ± 2.05	-
8	**32**	77.18 ± 1.73	26.07
9	**33**	80.19 ± 1.32	25.46
10	**34**	82.26 ± 1.46	381.76

The target phenothiazinyltetrahydropyrimidine carboxamide **29** possessing no substituents on the aryl group of amide unit exhibited 83% inhibition of DPPH radical when compared with the inhibition by ascorbic acid, the standard. Incorporation of chloro moieties on the meta- and para- positions of the aryl moiety of amide unit in **29** (i.e., in **25**) reduced the inhibition percentage to a magnitude of 8%, however, integration of nitro group on the meta- position of the aryl moiety of amide unit in **29** (i.e., in **26**) reduced the inhibition percentage to a magnitude of only 2%. In addition, the carboxamide containing methoxy substituents on the meta- and para- positions **(27)**, fluoro substituent on the ortho- position **(30)**, methyl substituents on the meta- and para- positions **(31)** and bromo substituent on the para position **(32)** of the aryl moiety of amide unit showed almost similar DPPH inhibition when compared with the molecule possessing chloro substituents on the meta- and para- positions of the aryl moiety of amide unit **(25)**. On the other hand, the molecules possessing bromo substituent on the ortho- position of the aryl unit **(33)** and methyl substituents on the ortho- and para- positions of the aryl moiety **(34)** exhibited almost similar DPPH inhibition when compared with the one possessing nitro substituent on the meta- position of the aryl unit of amide scaffold **(26)**. Among all the phenothiazinyltetrahydropyrimidine carboxamides synthesized, the one tethered with fluoro substituent on the para- position of the aryl moiety of the amide scaffold **(28)** exerted highest DPPH inhibition (87%).

Anticancer activity

One of the deadliest maladies in the globe is cancer and, there are numerous varieties of cancers which affect human. While several categories of drugs have been developed to combat cancers, the discovery of newer drugs for the same is vital as there are no precise drugs which could entirely cure the cancer is existing. Also, the drugs, in common, would become resistant over a period of time because of the environmental / biological variations. In this piece of research, the studies on cytotoxicity of the entire target phenothiazinyltetrahydropyrimidine carboxamides **25-34** have been assessed against AsPC1 and SW1990, the pancreatic cancer cell lines by the methodology of Cell-Titre Glo Luminescent Cell Viability Assay.[44-48] Against the tested carboxamides, the survival percentages of pancreatic cancer cells are furnished in **Table 3**.

The carboxamides possessing ethyl substituent on the 10-position of phenothiazine unit and chloro functionalities on the meta- and para- positions of the aryl moieties of amide scaffold **(25)**, nitro group on the meta- position of amide scaffold **(26)**, methoxy substituents on the meta- and para-positions of the amide moiety **(27)**, fluoro substituent on the para- position of the amide unit **(28)** and methyl substituent on the meta- and para- positions of the amide scaffold **(31)** exerted IC_{50} value of greater than 100 μM against both the cells investigated. The molecules **32** and **33** showed better activity against the cell SW1990 while the molecule **34** exhibited superior activity against both the cells ASPC1 and SW1990. Among all the molecules tested, the one possessing ethyl substituent on the nitrogen of the phenothiazine and no substituents on the amide unit **(29)** showed the best activity against both the cancer cells tested. The activity of all the molecules against a normal cell MRC5 is also investigated and found that the IC_{50} against the most active molecule was much higher. This result explicitly implies that the most active molecule, the one possessing ethyl substituent on the nitrogen of the phenothiazine and no substituents on the amide unit **(29)** is less toxic.

Table 3. Cell viability of pancreatic cancer cells**.**

S.No.	Compound	IC_{50} μM		
		MRC5	ASPC1	SW1990
1	**25**	>100	>100	>100
2	**26**	>100	>100	>100
3	**27**	>100	>100	>100
4	**28**	>100	>100	>100
5	**29**	83.7 ± 1.80	15.7 ± 2.29	22.8 ± 4.24
6	**30**	84.7 ± 4.44	>100	>100
7	**31**	>100	>100	>100
8	**32**	>100	>100	82.7 ± 5.67
9	**33**	70.7 ± 3.11	>100	51.1 ± 3.31
10	**34**	92.6 ± 3.12	57.0 ± 4.12	76.0 ± 3.19

Molecular docking

The molecular sources of communications among the active sites of BCL-2 (B-Cell Lymphoma 2) and binding affinity, obtained from the protein data bank, and the structure of carboxamide **29**, a three dimensionally stabilized one (conformationally stabilized), were assessed via molecular docking studies by employing Auto dock version 4.0. The results harvested through docking are given in **Figure 6**.

The phenothiazinyltetrahydropyrimidine carboxamide **29** exhibited its binding energy -8.84 Kcal/mol in addition to predicted IC_{50} value of 368.23 nM. The chemical entity **29** show van der Waals communication with phenyl alanine, hydrophobic side chain possessing amino acid, glutamic acid, electrically charged side chain containing amino acid and glycine. Further, a conventional hydrogen bond interaction between an amino acid with electrically charged side chain aspartic acid and NH of the pyrimidine core was observed.

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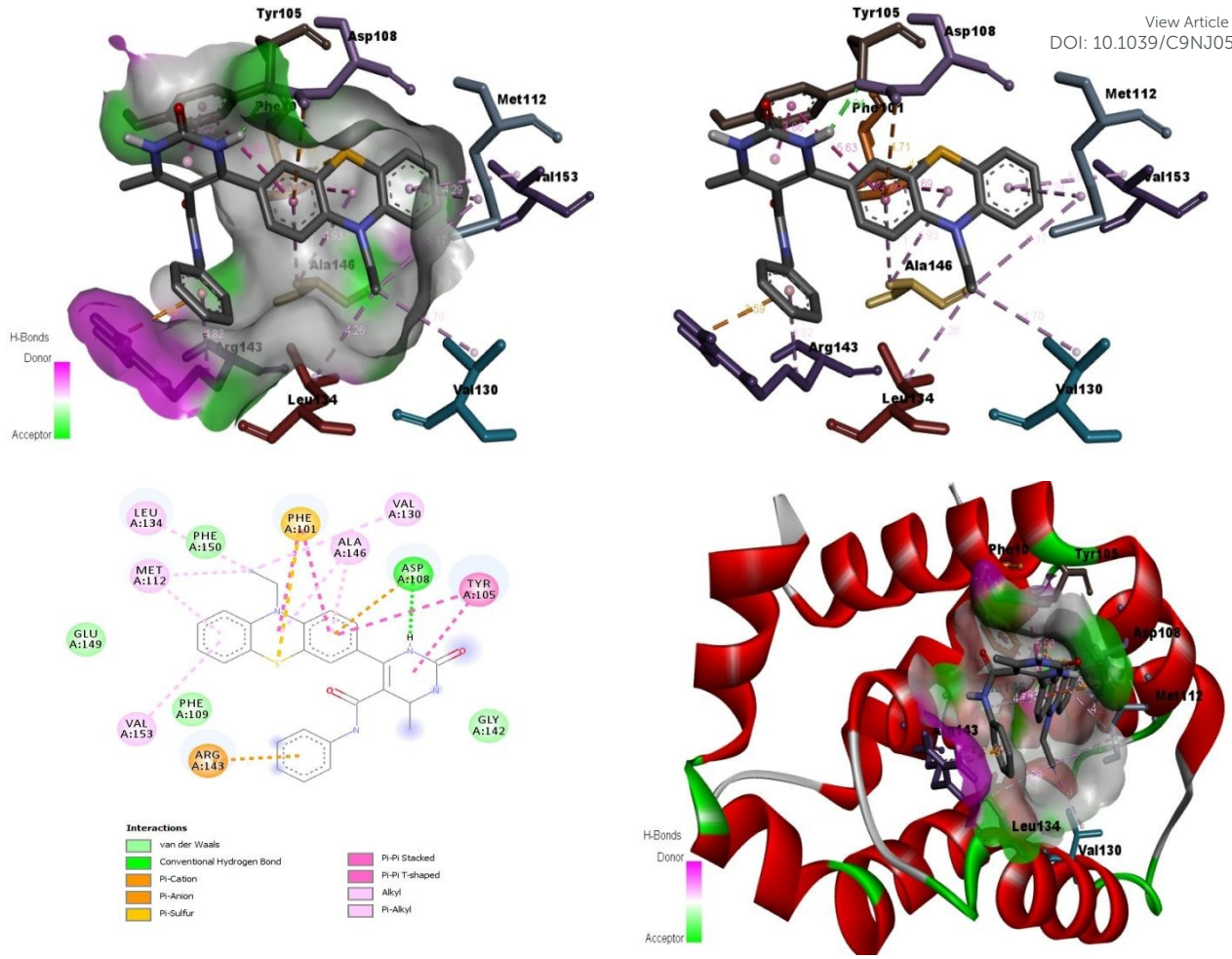

Fig 6. 2D & 3D interactions of BCL-2 with **29**.

Besides, an interaction between an amino acid with hydrophobic side chain phenyl alanine and sulphur present in the central ring of phenothiazine structural motif was also noted. In addition, there exist π-π stacked interactions between aromatic unit of tyrosine, an amino acid with hydrophobic side chain and one of the aromatic rings of the phenothiazine structural core as well as pyrimidine core of the molecule. It has also been observed various either alkyl-alkyl interactions or π-alkyl interactions between valine and methionine, amino acids with hydrophobic side chains and one of the aryl rings of the phenothiazine structural scaffold, valine, leucine and methionine, amino acids with hydrophobic side chains and methyl group of the ethyl moiety integrated at nitrogen of the phenothiazine, and also alanine, an yet other amino acid with hydrophobic side chain with another one aryl ring as well as central ring of the phenothiazine scaffold.

Conclusions

A series of phenothiazinyltetrahydropyrimidine carboxamides **25-34** has been synthesized through multi-step synthetic strategy. All the chemical entities have been characterized using physical and spectroscopic methods. Anti-inflammatory studies employing protein denaturation technique reveal that the target hybrid molecules integrated with m-nitro, p-fluoro, *m,p*-dimethyl, *o*-bromo and *o,p*-dimethyl phenyl carboxamide structural motifs exhibited enhanced activity than that of the standard diclofenac sodium under the experimental conditions. Radical scavenging activity through DPPH method reflects that p-fluorophenyl carboxamide tethered hybrid **(28)** showed the highest activity among the molecules investigated. Further, anticancer evaluation against pancreatic cancer cell lines SW1990 and AsPCl utilizing cell-titre glo luminescent cell viability assay methodology disclose that the hybrid integrated with phenyl carboxamide scaffold **(29)** provided the highest activity while comparing the rest of the molecules. Molecular docking revealed appreciable binding affinity between the most active chemical entity **29** and B-cell lymphoma 2. The results expose that these chemical entities could serve as potent biological agents and / or serve as efficient intermediates for the construction of potent biological agents. Further, anticancer exploration of the potent molecule with varied cell lines, besides development of structurally diversified significant hybrids which exert noticeable outcomes are currently under the way.

Experimental Section:

General

All Chemicals were procured from commercial suppliers as reagent grade and utilized as received. By adopting standard methods, all solvents were distilled/dried before their utilization. Pre-coated TLC sheets (silica-gel) were utilized for TLC and visualized by short- and long-wavelength UV lamps. Column chromatography was executed on silica-gel (spherical, 100–200 mesh) slurry packed in glass columns. The eluents utilized for individual separations are provided in the respective experimental protocols. All FT-IR spectra (KBr pellet form) were recorded in the range of 4000–400 cm^{-1} on a Shimadzu IR Tracer-100 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on NMR spectrometer (Bruker AVANCE II 400 and 100 MHz) at 25 °C with the use of TMS as an internal standard and DMSO-d_6 / $CDCl_3$ as solvent and chemical shifts are denoted in terms of ppm (δ ppm). The spin multiplicities m, q, t, d and s stand for multiplet, quartet, triplet, doublet, and singlet, respectively.

General method for the synthesis of 3-oxo-N-substituted phenylbutanamides (12-21)

To a solution of ethylacetoacetae (2 g, 15.4 mmol) in toluene (20 mL), was added powdered KOH (15 mol %). Respective substituted anilines **2-11** (17.0 mmol) were then added to the solution. The reaction mass was heated so as to reflux and it was continued (8 h) while stirring. Then it was extracted with DCM after attaining ambient temperature, and washed with aqua. The organic layer thus obtained was dried over Na_2SO_4 (anhyd.) and evaporated. The crude mass thus obtained was subjected to silica-gel column chromatography utilizing 10~20% ethyl acetate in hexane to eventually provide the respective substituted arylbutanamides **12-21**.[39]

Synthesis of alkylphenothiazine 23

In a characteristic experiment, an RB flask (250 mL) was charged with *N, N*-dimethylformamide (50 mL) and 10*H*-phenothiazine (0.025 mol). Powdered KOH (0.03 mol) was then added into the reaction vessel and it was stirred at ambient temperature for 2h. To this mixture, bromoethane (0.04 mol) was added (drop-wise) using a pressure equalizer for 20 min. and the reaction was continued at the same temperature for 24 hrs. The reaction progress was examined through TLC. The mixture was then extracted with DCM (3 x 20 mL), washed well with aqua and dried over Na_2SO_4 (anhyd.). Removal of the DCM and subsequent purification through column chromatography eventually offered the title compound **23**.[40]

Synthesis of carbaldehyde 24

In a two neck RB flask (100 mL) under inert atmosphere, was charged DMF (1 mL). $POCl_3$ (1.2 mL) was then added in a drop-wise fashion at 0 °C and stirred for 45 minutes. A chloroform (20 mL) solution of phenothiazine **23** (11.0 mmol) was then added drop wise into the reaction mixture. After the addition is completed, the temperature was increased to 60 °C and the stirring was continued for 6 hrs. The reaction progress was monitored through TLC. The reaction mixture, after completion of the reaction, was extracted with DCM (3 × 20 mL), washed well with aqua, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude thus resulted was subjected to column chromatography to furnish the pure product **24**.[40]

General method for the synthesis of hybrid heterocycles (25-34):

To an ethanolic mixture of carbaldehyde **24** (1 equi. in 15 mL), were added respective substituted phenyloxobutanamides **12-21** (1.1 equi.), urea (1.5 equi.) and catalytic amount of *p*-toluenesulfonic acid (15 mol %). The mass in the flask was refluxed for 12 h and the solvent was evaporated utilizing rotary evaporator after attaining ambient temperature. The crude thus obtained was then extracted with DCM after diluting with aqua. The organic layer was separated, dried on Na_2SO_4, and evaporated using a rotary evaporator. The solid thus resulted was subjected to column chromatography using the eluent system ethyl acetate in hexane (40~50 %) to provide respective pure target hybrid molecules **25-34** as solids.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 25

To a mixture of carbaldehyde **24** (0.5 g, 1.96 mmol) in ethanol (15 ml), was added aryloxobutanamide **12** (0.53 g, 2.15 mmol), Urea (0.18 g, 2.94 mmol) and catalytic amount of *p*-toluenesulfonic acid (10 mol%). After completion of the reaction by adopting the general method, it gave the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **25** (hexane-ethyl acetate 6:4). Yield, 0.92g (89%); MP: 124-126°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, 439.7; ^1H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.56-6.71 (m, 11H), 6.38 (s, 1H), 5.19 (s, 1H), 3.78 (br.d, *J*=6.02 Hz, 2H), 2.15 (s, 3H), 1.29 (t, *J*=6.80 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 153.5, 145.7, 144.1, 140.2, 137.3, 136.1, 132.5, 130.3, 127.5, 125.3, 122.7, 121.6, 119.2, 115.4, 115.2, 105.3, 55.9, 41.9, 17.9, 13.1; Anal. Calcd. for $C_{26}H_{22}Cl_2N_4O_2S$ (%): C, 59.43; H, 4.22; N, 10.66; S, 6.10; Found: C, 59.63; H, 4.33; N, 10.49; S, 6.02.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 26

A mixture of carbaldehyde **24** (0.5 g, 1.96 mmol), aryloxobutanamide **13** (0.48 g, 2.15 mmol), urea (0.18 g, 2.94 mmol) and catalytic amount of *p*-toluenesulfonic acid (10 mol%) in ethanol provided the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **26** (hexane-ethyl acetate 6:4). Yield, 0.85g (86%); MP: 142-144°C; FT-IR (KBr, cm^{-1}): ν 3240.4, 2956.8, 2926.0, 1598.9, 1529.5, 1463.9, 1425.4, 1384.8,1350.1, 1327.0, 1236.0, 1134.1, 1107.1, 1087.8, 1043.4, 945.1, 881.4, 815.8, 796.6, 736.8, 702.0, 671.2, 596.0, 538.1, 453.2, 418.5; ^1H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.93-6.74 (m, 12H), 6.22 (s, 1H), 5.27 (s, 1H), 3.81 (br.d, *J*=6.00 Hz, 2H), 2.24 (s, 3H), 1.31 (t, *J*=6.60 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.3, 153.1, 145.7, 145.5, 143.5, 134.8, 132.6, 130.5, 130.0, 128.4, 127.9, 127.4, 120.3, 118.9, 118.8, 105.4, 55.9, 41.9, 17.8, 13.1; Anal. Calcd. for $C_{26}H_{23}N_5O_4S$ (%): C, 62.26; H, 4.62; N, 13.96; S, 6.39; Found: C, 62.43; H, 4.73; N, 13.84; S, 6.30.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 27

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To a mixture of carbaldehyde **24** (0.5 g, 1.96 mmol) in ethanol (15 ml), was added aryloxobutanamide **14** (0.51 g, 2.15 mmol), urea (0.18 g, 2.94 mmol) and catalytic amount of *p*-toluenesulfonic acid (10 mol%). After completion of the reaction by adopting the general method, it gave the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **27** (hexane-ethyl acetate 6:5). Yield, 0.83g (82%); MP: 152-153°C; FT-IR (KBr, cm^{-1}): ν 3230.7, 3115.0, 2960.7, 2908.6, 2835.3, 1699.2, 1670.3, 1624.0, 1602.8, 1512.1, 1467.8, 1442.7, 1411.8, 1382.9, 1363.6, 1330.8, 1244.0, 1201.6, 1166.9, 1134.1, 1022.2, 964.4, 881.4, 844.8, 800.4, 752.2, 713.6, 653.8, 597.9, 543.9, 513.0, 489.9; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78-6.66 (m, 12H), 5.60 (s, 1H), 5.25 (s, 1H), 3.88 (br.d, *J*=6.05 Hz, 2H), 3.81 (s, 3H), 3.76 (s, 3H), 2.23 (s, 3H), 1.28 (t, *J*=6.72 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.3, 153.6, 145.9, 138.8, 131.1, 127.5, 125.8, 125.4, 122.7, 115.5, 112.0, 111.2, 105.0, 56.3, 56.1, 55.8, 41.9, 18.0, 12.9; Anal. Calcd. for $C_{28}H_{28}N_4O_4S$ (%): C, 65.10; H, 5.46; N, 10.85; S, 6.21; Found: C, 64.93; H, 5.58; N, 10.70; S, 6.11.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 28

A mixture of carbaldehyde **24** (0.5 g, 1.96 mmol), aryloxobutanamide **15** (0.42 g, 2.15 mmol), urea (0.18 g, 2.94 mmol) and catalytic amount of *p*-toluenesulfonic acid (10 mol%) in ethanol provided the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **28** (hexane-ethyl acetate 6:5). Yield, 0.81g (87%); MP: 188-189°C; FT-IR (KBr, cm^{-1}): ν 3435.2, 3373.5, 3215.3, 3089.9, 1710.8, 1653.0, 1624.0, 1510.2, 1463.9, 1404.1, 1328.9, 1236.3, 1201.6, 1159.2, 1132.2, 1078.2, 1039.6, 1012.6, 941.2, 831.3, 810.1, 794.6, 761.8, 746.4, 702.0, 677.0, 657.7, 609.5, 580.5, 549.7, 511.1, 493.7, 453.2; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-6.81 (m, 13H), 5.58 (s, 1H), 5.24 (s, 1H), 3.89 (br.d, *J*=6.00 Hz, 2H), 2.24 (s, 3H), 1.39 (t, *J*=6.80 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7, 153.0, 144.7, 144.0, 139.0, 138.9, 135.9, 128.0, 127.4, 125.9, 123.4, 122.7, 121.8, 115.5, 115.3, 105.5, 54.6, 41.5, 17.5, 13.1; Anal. Calcd. for $C_{26}H_{23}FN_4O_2S$ (%): C, 65.81; H, 4.89; N, 11.81; S, 6.76; Found: C, 65.98; H, 4.99; N, 11.69; S, 6.70.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 29

To a mixture of carbaldehyde **24** (0.5 g, 1.96 mmol) in ethanol (15 ml), was added aryloxobutanamide **16** (0.38 g, 2.15 mmol), urea (0.18 g, 2.94 mmol) and catalytic amount of *p*-toluenesulfonic acid (10 mol%). After completion of the reaction by adopting the general method, it gave the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **29** (hexane-ethyl acetate 6:4). Yield, 0.79 (88%); MP: 138-140°C; FT-IR (KBr, cm^{-1}): ν 3224.9, 3103.4, 2933.7, 1697.3, 1597.0, 1525.9, 1496.7, 1463.9, 1438.9, 1384.8, 1367.5, 1325.1, 1238.3, 1195.8, 1134.1, 1111.0, 1091.7, 1074.3, 1039.6, 889.8, 750.3, 692.4, 663.5, 599.8, 530.4, 455.2; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26-6.81 (m, 14H), 5.53 (s, 1H), 5.25 (s, 1H), 3.89 (br.d, *J* = 5.81 Hz, 2H), 2.24 (s, 3H), 1.39 (t, *J* = 6.60 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 153.0, 144.7, 144.0, 139.6, 138.9, 129.1, 128.9, 127.4, 126.0, 125.3, 123.5, 122.8, 120.0, 115.8, 115.7, 105.7, 54.7, 41.5, 17.5, 13.1; Anal. Calcd. for $C_{26}H_{24}N_4O_2S$ (%): C, 68.40; H, 5.30; N, 12.27; S, 7.02; Found: C, 68.59; H, 5.47; N, 12.11; S, 6.90.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 30

A mixture of carbaldehyde **24** (0.5 g, 1.96 mmol), aryloxobutanamide **17** (0.42 g, 2.15 mmol), urea (0.18 g, 2.94 mmol) and catalytic amount of *p*-toluenesulfonic acid (10 mol%) in ethanol provided the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **30** (hexane-ethyl acetate 6:5). Yield, 0.85g (91%); MP: 168-170°C; FT-IR (KBr, cm^{-1}): ν 3340.7, 3209.5, 2960.7, 2924.0, 2854.6, 1697.3, 1662.6, 1627.9, 1523.7, 1500.6, 1458.1, 1319.3, 1261.4, 1022.2, 910.4, 800.4, 748.3, 702.0, 663.5, 605.6, 578.6, 553.5, 532.3, 486.0, 451.3; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (s, 1H), 7.40-6.71 (m, 12H), 5.47 (s, 1H), 5.16 (s, 1H), 3.83 (q, *J*=6.67 Hz, 2H), 2.24 (s, 3H), 1.32 (t, *J* = 6.60 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.4, 152.6, 144.4, 143.4, 140.4, 135.0, 126.3, 125.3, 124.9, 124.6, 124.4, 123.4, 123.1, 122.7, 121.5, 120.8, 114.5, 113.9, 103.5, 54.9, 40.8, 17.15, 11.92; Anal. Calcd. for $C_{26}H_{23}FN_4O_2S$ (%): C, 65.81; H, 4.89; N, 11.81; S, 6.76; Found: C, 65.99; H, 4.80; N, 11.69; S, 6.63.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 31

To a mixture of carbaldehyde **24** (0.5 g, 1.96 mmol) in ethanol (15 ml), was added aryloxobutanamide **18** (0.44 g, 2.15 mmol), urea (0.18 g, 2.94 mmol) and catalytic amount of *p*-toluenesulfonic acid (10 mol%). After completion of the reaction by adopting the general method, it gave the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **31** (hexane-ethyl acetate 6:4). Yield, 0.85g (89%); MP: 220-222°C; FT-IR (KBr, cm^{-1}): ν 3396.6, 3319.4, 3219.1, 3091.8, 1701.2, 1672.2, 1618.2, 1535.3, 1500.6, 1462.0, 1384.8, 1325.1, 1284.5, 1284.5, 1238.3, 1199.7, 1165.0, 1132.2, 1107.1, 954.7, 893.0, 864.1, 823.6, 744.5, 700.1, 675.0, 651.9, 592.1, 565.1, 536.2, 513.0, 457.1; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (s, 1H), 7.26-6.80 (m, 11H), 5.53 (s, 1H), 5.24 (s, 1H), 3.89 (br.d, *J*=6.80 Hz, 2H), 2.21 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 1.38 (t, *J*=6.80 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.5,153.0, 144.8, 144.0, 139.0, 137.3, 136.4, 131.2, 129.7, 128.0, 127.4, 126.0, 125.3, 123.8, 122.7, 121.3, 117.6, 115.8, 114.7, 105.8, 54.7, 41.5, 20.0, 19.2, 17.5, 13.1; Anal. Calcd. for $C_{28}H_{28}N_4O_2S$ (%): C, 69.40; H, 5.82; N, 11.56; S, 6.62; Found: C, 69.57; H, 5.96; N, 11.41; S, 6.50.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 32

A mixture of carbaldehyde **24** (0.5 g, 1.96 mmol), aryloxobutanamide **19** (0.55 g, 2.15 mmol), urea (0.18 g, 2.94 mmol) and catalytic amount of *p*-toluenesulfonic acid (10 mol%) in ethanol provided the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **32** (hexane-ethyl acetate 6:4). Yield, 0.91g (87%); MP: 114-116°C; FT-IR (KBr, cm^{-1}): ν 3288.6, 3103.4, 2958.8, 2924.0, 2854.6, 1753.9, 1701.2, 1653.0, 1624.0, 1585.4, 1502.5, 1463.9, 1390.6, 1330.8, 1236.3, 1197.7, 1136.0, 1109.0, 1010.7, 902.6, 817.8, 748.3, 700.1, 675.0, 601.7, 565.1, 501.4, 464.8, 428.2; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51 (s, 1H), 7.32-6.79 (m, 12H), 5.71 (s, 1H), 5.21 (s, 1H), 3.87 (br.d, *J*=6.00 Hz, 2H), 2.22 (s, 3H), 1.37 (t, *J*=6.80 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8, 152.9, 144.8, 143.2, 135.7, 130.7, 126.4, 124.6, 122.3, 121.6, 120.5,

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115.7, 114.3, 104.1, 54.8, 40.8, 16.8, 13.1; Anal. Calcd. for $C_{26}H_{23}BrN_4O_2S$ (%): C, 58.32; H, 4.33; N, 10.46; S, 5.99; Found: C, 58.51; H, 4.42; N, 10.33; S, 5.90.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 33

To a mixture of carbaldehyde **24** (0.5 g, 1.96 mmol) in ethanol (15 ml), was added aryloxobutanamide **20** (0.55 g, 2.15 mmol), urea (0.18 g, 2.94 mmol) and catalytic amount of *p*-toluenesulfonic acid (10 mol%). After completion of the reaction by adopting the general method, it gave the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **33** (hexane-ethyl acetate 6:5). Yield, 0.86g (82%); MP: 184-186°C; FT-IR (KBr, cm^{-1}): ν 3402.4, 3344.5, 3213.4, 2962.6, 1695.4, 1668.4, 1602.8, 1514.1, 1465.9, 1367.5, 1261.4, 1220.9, 1091.7, 1020.3, 902.6, 802.3, 750.3, 705.9, 665.4, 549.7, 511.1, 464.8, 441.7; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.39-6.77 (m, 12H), 5.50 (s, 1H), 5.19 (s, 1H), 3.82 (br.d, *J*=6.40 Hz, 2H), 2.27 (s, 3H), 1.32 (t, *J*=6.80 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.0, 152.7, 144.7, 141.8, 137.1, 132.7, 131.9, 131.1, 130.2, 129.5, 128.5, 127.3, 120.3, 119.9, 119.8, 103.4, 54.8, 40.6, 17.0, 11.9; Anal. Calcd. for $C_{26}H_{23}BrN_4O_2S$ (%): C, 58.32; H, 4.33; N, 10.46; S, 5.99; Found: C, 58.47; H, 4.40; N, 10.36; S, 5.91.

Hybrid phenothiazinyltetrahydropyrimidinecarboxamide 34

A mixture of carbaldehyde **24** (0.5 g, 1.96 mmol), aryloxobutanamide **21** (0.44 g, 2.15 mmol), urea (0.18 g, 2.94 mmol) and catalytic amount of p-toluenesulfonic acid (10 mol%) in ethanol provided the target hybrid phenothiazinyltetrahydropyrimidinecarboxamide **34** (hexane-ethyl acetate 6:4). Yield, 0.87g (92%); MP: 198-200°C; FT-IR (KBr, cm^{-1}): ν 3394.7, 3203.7, 3105.3, 2972.3, 2825.7, 1705.0, 1676.1, 1631.7, 1498.6, 1462.0, 1359.8, 1328.9, 1244.0, 1201.6, 1159.2, 1112.9, 1041.5, 945.1, 898.8, 806.2, 752.2, 653.8, 613.3, 574.7, 549.2, 509.2, 453.2, 422.4; 1H NMR (400 MHz, $CDCl_3$): δ 7.48 (s, 1H), 7.26-6.59 (m, 11H), 5.40 (s, 1H), 5.25 (s, 1H), 3.89 (br.d, J=6.80 Hz, 2H), 2.28 (s, 3H), 2.23 (s, 3H), 2.17 (s, 3H), 1.39 (t, J=6.80 Hz, 3H); 13C NMR (100 MHz, CDCl3): δ 164.9, 152.9, 145.3, 144.5, 136.0, 134.8, 132.8, 131.3, 127.4, 126.0, 125.7, 123.5, 122.6, 115.1, 104.9, 56.1, 41.9, 17.3, 12.9; Anal. Calcd. for C28H28N4O2S (%): C, 69.40; H, 5.82; N, 11.56; S, 6.62; Found: C, 69.58; H, 5.99; N, 11.41; S, 6.54.

Anti-inflammatory activity by Protein denaturation method

For the anti-inflammatory (*in vitro*) study, the method adopted in the literature was used with slight modification.[41,42] The reaction mixture (2.5 mL) consisted of egg albumin (0.1 mL), synthesized molecule (1 mL; 1 mM) and phosphate buffered saline (PBS, 1.4 mL; pH 6.4). Equal volume of water (double distilled) served as control. 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 vehicle as blank. As a reference drug, diclofenac sodium (1 mM) was utilized and treated likewise for the absorbance determination. The inhibition (%) of protein denaturation was then calculated.

Radical scavenging activity by DPPH method

The assay of radical scavenging (DPPH) has been performed using the method furnished in the literature[43] with small modifications. Firstly, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (1.6 mg) was dissolved in DMSO (50 mL). To each compound prepared (1.5 mL, 100 µg/mL), was added DPPH solution (1.5 mL) and set aside for 45 min incubation under dark condition at ambient temperature. The variations in absorbance were then noted (517 nm). The absorbance of the blank DPPH solution was used as control (517 nm). The DPPH free radical scavenging activity was then calculated.

Anticancer evaluation

Human pancreatic cancer cells AsPC1 and SW1990 were purchased from ATCC, USA. RPMI-FBS medium supplemented with 100 µg/mL of streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and 100 IU/mL of penicillin was used to culture all cells. All pancreatic cancer cells were incubated at 37 °C in 5% CO2, utilizing the standard cell culture method (in vitro) established previously.[44-48] Gemcitabine was used as a positive control.

With a view to assess antitumor effects of the synthesized molecules, stock solutions of the same in DMSO (100 mM) were initially prepared, then further diluted to 0.1 mM in sterile PBS. As reported in the literature,[44,49] the cells were treated on the basis of cell viability assay. Firstly, 384-well plates were seeded with cells in such a way that each well contained 1,500 cells/well. Plates were incubated for 24 hours and then the cells were treated with the synthesized molecules for 72 hours. 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).

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of treated (with compounds)}}{\text{Absorbance of untreated}} \text{ X } 100$$

Conflicts of interest

The authors declare no conflict of interest.

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Phenothiazine and amide ornamented novel nitrogen heterocyclic hybrids: Synthesis and their biological and molecular docking studies

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