Journal Pre-proofs

Anti-proliferative Potential of Triphenyl Substituted Pyrimidines Against MDA-MB-231, HCT-116 and HT-29 cancer cell lines

Ashish Ranjan Dwivedi, Vijay Kumar, Harmeet Kaur, Naveen Kumar, Ravi Prakash Yadav, P Ramarao, Somesh Baranwal, Vinod Kumar

PII: S0960-894X(20)30579-5
DOI: https://doi.org/10.1016/j.bmcl.2020.127468
Reference: BMCL 127468

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 15 May 2020
Revised Date: 26 July 2020
Accepted Date: 3 August 2020

Please cite this article as: Ranjan Dwivedi, A., Kumar, V., Kaur, H., Kumar, N., Prakash Yadav, R., Ramarao, P., Baranwal, S., Kumar, V., Anti-proliferative Potential of Triphenyl Substituted Pyrimidines Against MDA-MB-231, HCT-116 and HT-29 cancer cell lines, *Bioorganic & Medicinal Chemistry Letters* (2020), doi: https://doi.org/10.1016/j.bmcl.2020.127468

Anti-proliferative Potential of Triphenyl Substituted Pyrimidines Against MDA-MB-231, HCT-116 and HT-29 cancer cell lines

Ashish Ranjan Dwivedi[a], Vijay Kumar[a], Harmeet Kaur[a], Naveen Kumar[a], Ravi Prakash Yadav[b], P Ramarao[a], Somesh Baranwal[b*] and Vinod Kumar[a,c*]

[a]*Department of Pharmaceutical Sciences and Natural Products, Central University of Punjab, Bathinda, Punjab, India-151001.*

[b]*Department of Microbiology, School of Basic and Applied Sciences, Central University of Punjab, Bathinda, Punjab, India-151001.*

[c]*Laboratory of Organic and Medicinal Chemistry, Department of Chemistry, Central University of Punjab, Bathinda, Punjab, India-151001.*

Corresponding Author:

***Dr Vinod Kumar**

Laboratory of Organic and Medicinal Chemistry, Department of Chemistry, Central University of Punjab, Bathinda, Punjab, India-151001.

E-mail: vpathania18@gmail.com; vinod.kumar@cup.edu.in

Phone No. +911642864214

Co-corresponding author:

Dr. Somesh Baranwal

Department of Microbiology, School of Basic and Applied Sciences, Central University of Punjab, Bathinda, Punjab, India

E-mail: someshbaranwal@gmail.com; somesh.baranwal@cup.edu.in

Conflict of Interest Statement: Authors declare no potential conflict of interest.

Abstract

A series of triphenyl substituted pyrimidines as analogous of colchicine and combretastatin A-4 was synthesized and evaluated for the antiproliferative potential. The compounds were screened against MDA-MB-231, HCT-116 and HT-29 cell lines using MTT assay. Most of the compounds displayed antiproliferative activity in low to sub micro molar concentration. Amongst the synthesized derivatives, compounds **HK-2, HK-10 and HK-13** were found to be effective against all the three cancer cell lines. **HK-2** exhibited IC_{50} values of 3.39 μM, 4.78 μM and 4.23 μM, **HK-10** showed IC_{50} values of 0.81 μM, 5.89 μM, 4.96 μM and **HK-13** showed IC_{50} values 3.24 μM, 4.93 μM and 4.73 μM against MDA-MB-231, HCT-116 and HT-29 cancer cell lines, respectively. **HK-10** was found to be the most potent compound in the series with IC_{50} values of 0.81 μM against MDA-MB-231. In the cell cycle analysis, **HK-2** and **HK-10** showed cell arrest at G2/M phase of the cell cycle while **HK-13** inhibited cell growth at the G1/G0 phase. All the three compounds showed cell death induced through apoptosis. In the docking studies, **HK-2, HK-10** and **HK-13** were found to fit well in the colchicine binding site of the tubulin. Some of the compounds in the current series were found to be promising against all the three cancer cell lines and may act as potent leads for further development.

Keywords: Antiproliferative agents, Pyrimidines, Colchicine binding site, Tubulin polymerization inhibitors, Combretastatin

Cancer is one the leading cause of death all over the world. Despite of continuous advancements in the treatment and prevention strategies, the successful treatment of cancer, in particular metastases, still remains a big challenge.[1] Discovery of new and safer anticancer agents that are more potent and effective with improved cytotoxicity in cancer cells would improve the management of cancer.[2] In 2018, the disease led to nearly 700,000 deaths in India.[3] Cancer is a multifactorial disease and a number of cellular pathways are associated with the progression of the disease. Major problems associated with the cancer treatment are toxicity to the normal cells and development of resistance to therapy.[4] More safe and effective anticancer ligands that target EGFR (Epidermal Growth Factor Receptors), VEGF (Vascular Endothelial Growth Factor), PARP (poly ADP ribose polymerase), telomerase, and tubulin are being developed.[5-8]

Cancer cell has the ability to evade apoptosis, a programmed cell death, and microtubule targeting drugs may trigger apoptosis by disrupting the tubulin-microtubule equilibrium.[9] Microtubules are composed of α,ß-tubulin heterodimers that play crucial role in cell division, intracellular trafficking, cell migration and angiogenesis.[10] These tubulin polymers exist in dynamic equilibrium through polymerization and depolymerization processes. Numerous structurally different natural as well as synthetic compounds have been identified that target microtubule polymerization. Colchicine, a natural product, bind to the colchicine binding site of the tubulin and inhibit tubulin polymerization. Similarly, combretastatin A-4 (CA-4) also bind to the colchicine binding site and disturb the tubulin-microtubule equilibrium by inhibiting tubulin polymerization. Thus, the colchicine binding site (CBS) present on the tubulin is being explored as a promising target for developing new drugs for the treatment of cancer.[11-13] All the CBS ligands inhibit the cell division in G-2/M phase of the cell cycle and causes cell death either through necrosis or apoptosis.[14] Most of the CBS ligands are made up of three fragments having two binding groups and a bridge.[15] CA-4, a potent microtubule targeting and vascular damaging agent, is capable of drastically inhibiting cancer cell proliferation *in vitro*.[16] CA-4P, a comparatively safer phosphate derivative of CA-4 is under clinical trials and it has revealed its ability to regress tumor vasculature in variety of cancers.[17] CA-4P was granted fast track status by the FDA for the treatment of platinum-resistant ovarian cancer.[18-20] Thus, a large number of CA-4 derivatives containing different heterocyclic rings such as tetrazole, triazoles,[21, 22] imidazole,[23] pyrazole,[24] pyrimidine,[25] have been designed, synthesized and screened for the anticancer potential. However, clinical application of these agents is not successful till

now.[26] It is a big challenge to synthesize CA-4 analogues with improved activity along with therapeutic potency.

In the careful analysis of the approved drugs (FDA.gov2019), it has been observed that more than 60% of the approved anticancer drugs in 2019 including entrectinib, zanubrutinib etc contain nitrogen based heterocyclic compounds.[27] Pyrimidine represent one of the important nitrogen containing heterocycles present in many drugs such as imatinib and rosuvastatin.[28] The pyrimidine ring can be optionally substituted to synthesize different types of compounds with diverse pharmacological profile.[29] Various pyrimidine ring containing scaffolds such as imatinib, monastrol and ispinesib (Fig-1) have been explored as attractive agents for the cancer treatment.[30, 31] Lefebvre *et al.* screened 4-aryl-6-(piperidin-1-yl)pyrimidines with a sulphonamide group against different cancer cell lines and reported that the presence of a bulkier substituent on the aromatic ring has increased the inhibition potential of the compounds.[32] Czudor *et al.* synthesized a series of 2,4-disubstituted pyrimidine derivatives and tested against the Cyclin-dependent kinases (CDKs) and Polo-like kinases (PLKs). Most of these compounds displayed nanomolar activity against CDK9 and significant antiproliferative potential against multiple myeloma cell lines (RPMI-8226).[33] Zonghui *et al.*, developed a series of tetrahydropyrido[4,3-d]pyrimidine-2,4-dione based compounds that showed potent anticancer activity.[34] Similarly, Jin *et al.* designed and synthesized compounds based on phenylpyrimidine pharmacophore model. The lead compounds showed good activity against HepG2 and MCF-7 cancer cell lines.[35]

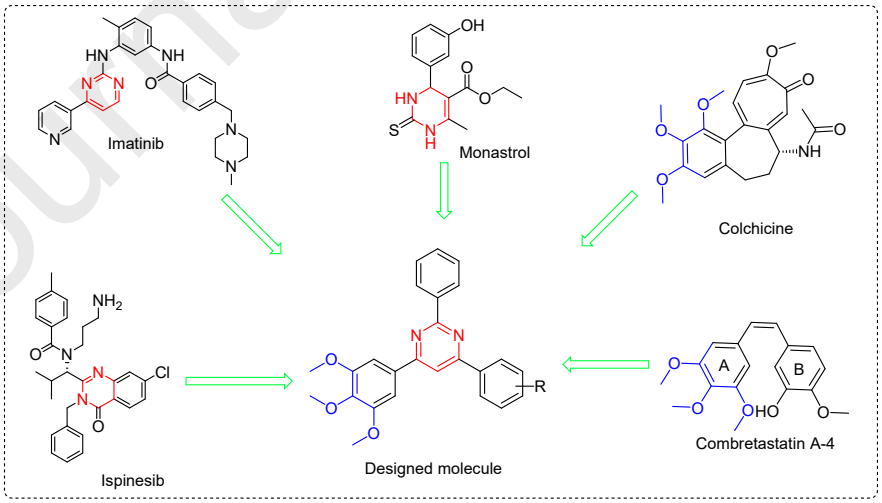
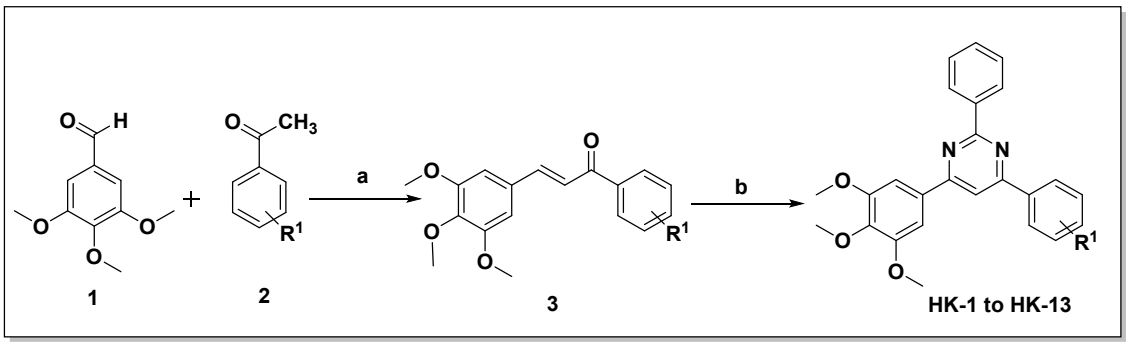


Fig-1, Target compounds and their structural comparison with colchicine, combretastatin A-4 (CA-4) and other pyrimidine derivatives.

Recently, we have synthesized and screened a series of CA-4 inspired biphenyl pyrimidines for their anticancer potential.[14] Most of the compounds displayed anticancer activity with IC_{50} values in low micro-molar range. Taking leads from our previous study and literature reports[14], in the current research work we decided to increase the bulk on the pyrimidine ring by incorporating additional phenyl ring at the C-2 position. 3,4,5-trimethoxyphenyl group was kept as ring A as it is reported to play crucial role in the binding of ligands to the colchicine binding site.[36] Pyrimidine ring act as linker while aromatic ring C was substituted with different electron withdrawing and electron releasing groups to develop structure-activity relationship profile. C-2 position of the pyrimidine ring was substituted with an aromatic ring to increase the bulk.[32] The synthesized compounds were screened against MDA-MB-231, HCT-116 and HT-29 cancer cell lines for their antiproliferative activity. Most of the compounds were found to be effective against all the three cancer cell lines and displayed IC_{50} values in low to sub micro molar range. HK-2, HK-10 and HK-13 were found to be the potent lead compounds against all the three cancer cell lines. HK-10 with IC_{50} values of 0.81± 0.11 µM, 5.89 ± 0.57 µM, 4.96 ± 0.39 µM against MDA-MB-231, HCT-116, HT-29 respectively, was found to be the most potent compound in the series and displayed comparable activity as compared to the standard colchicine. The molecular modelling studies of the potent compounds were also performed in order to understand the binding orientation and interactions of the compounds with the tubulin protein. The cell cycle and apoptosis analysis of the potent compounds revealed that these compounds kill the cancer cells in G-2/M phase of the cell cycle and cell death is caused due to apoptosis.

Target compounds were synthesized via two step procedure as described in **Scheme 1.** In the first step, the intermediate 1,3-diphenyl-2-propen-1-one chalcones (**3**) were synthesized through acid catalyzed Claisen-Schimdt condensation between trimethoxy substituted aldehydes **(1)** and different acetophenones **(2),** and the intermediates were recrystallized to obtain pure products.[37-39] In the second step, the intermediate chalcones **(3)** were reacted with benzamidine hydrochloride using sodium carbonate and acetonitrile to get corresponding pyrimidine bridged final products **(**HK-1 to HK-13**)**.[40] All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, ESI-MS and HRMS.[41-43]

Scheme 1. Synthesis of pyrimidine bridged analogues of combretastatins



Reagent and conditions: a) 10 mol % H_2SO_4 in aceticacid, strring, reflux **b)** Benzamidine hydrochloride (1.4 eq), anhyd Na_2CO_3 (2.5 eq), acetonitrile (10ml) , reflux,

All the synthesized compounds **(HK-1 to HK-13)** were investigated for their *in vitro* antiproliferative activity against human MDA-MB-231 (breast), HCT-116 (colon) and HT-29 (colon) cancer cell lines using standard MTT assays (Table 1) with colchicine as a reference compound. Four different concentrations (1 µM, 5 µM, 10 µM and 25 µM) of the test compounds were used and the results were analyzed after 48 h of drug treatment. Each concentration was evaluated in triplicate and the cells incubated without the test compounds were used as control. Cells viability was assessed and antiproliferative activity was calculated in terms of IC_{50} values recorded in Table 1. Most of the compounds displayed activity in low to sub micromolar range against all the three cancer cell lines. As evident from Table 1, compounds **HK-2, HK-3, HK-4, HK-10 and HK-13** were found effective against all the three cancer cell lines. **HK-10** was found to be the most potent against MDA-MB-231 cancer cell line with IC_{50} value of 0.81 µM. Similarly, **HK-6** was found to be the most potent against HCT-116 and HT-29 cancer cell lines with IC_{50} values of 4.32 µM and 3.17 µM, respectively. In all the three cases IC_{50} values of the test compounds were found to be better than the standard colchicine. In this series, **HK-2, HK-10 and HK-13** showed best antiproliferative activity against all the three cancer cell lines. Compound **HK-2** exhibited IC_{50} values of 3.39 µM, 4.78 µM and 4.23 µM against MDA-MB-231, HCT-116 and HT-29 cell lines respectively. Similarly, **HK-10** and **HK-13** showed IC_{50} values of 0.81 µM, 5.89 µM, 4.96 µM and 3.24 µM, 4.93 µM, 4.73 µM against MDA-MB-231, HCT-116, HT-29 cell lines, respectively (48 h post treatment). The antiproliferative activities of all the compounds, HK-1 to HK-13 and reference colchicine against MDA-MB-231, HCT-116 and HT-29 are described in Table 1.

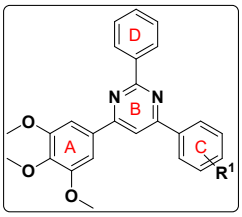


Table 1: Evaluation of *in vitro* antiproliferative activity (IC_{50} in µM) of compounds **HK-1 to HK-13** against MDA-MB-231, HCT-116 and HT-29 cancer cell lines (Data are presented as the mean ± SDs of three independent experiments)

Compound	C ring	R^1	IC_{50} (µM)		
			MDAMB231 (µM)	HCT116 (µM)	HT29(µM)
HK-1		4-CH_3	5.11 ± 0.13	15.67 ± 0.13	>25
HK-2		4-OCH_3	3.39 ± 0.06	4.78 ± 0.33	4.23 ± 0.16
HK-3		-H	5.87 ± 0.09	9.95 ± 0.51	7.93 ± 0.25
HK-4		4-OH	1.60 ± 0.37	11.85 ± 0.65	11.34 ± 0.18
HK-5		4-Cl	>25	7.98 ± 0.41	6.23 ± 0.15
HK-6		4-Br	>25	4.32 ± 0.21	3.17 ± 0.68
HK-7		-H	5.11 ± 0.49	16.97 ± 0.27	>25
HK-8		2,4-OCH_3	1.44 ± 0.08	>25	>25
HK-9		3-OH	1.99 ± 0.18	20.35 ± 1.58	>25
HK-10		2,4-OH	0.81 ± 0.11	5.89 ± 0.57	4.96 ± 0.19
HK-11		3-NO_2	2.44 ± 0.51	>25	>25
HK-12		4-NH_2	1.01 ± 0.13	12.34 ± 0.57	>25
HK-13		-H	3.24 ± 0.23	4.93 ± 0.46	4.73 ± 0.16

Colchicine		0.93 ± 0.13	5.13 ± 0.35	5.19 ± 0.42

Cell cycle and apoptosis studies of the lead compounds

HK-2, HK-10 and HK-13 have displayed promising antiproliferative activities and selected for cell cycle analysis and apoptosis studies to understand the mechanism of action of the compounds. This series of compounds are derived from colchicine and combretastatin A-4 and are expected to bind to the colchicine binding site of the tubulin. Ligands binding to the colchicine site, act as tubulin polymerization inhibitors and arrest the cell cycle at G2/M phase. Thus, the cell cycle analysis of these three compounds was performed on HCT-116 cancer cells. Cells were grown in 6 well-treated corning plate, and treated with 12.5 µM concentration of the compounds. It was found that HK-2, and HK-10 showed cell cycle arrest of 88.8 %, and 79.5 %, respectively at the G2/M phase while HK-13 showed cell cycle arrest of 78.5 % at G1/G0 phase. Colchicine was used as a positive control and as expected it showed cells arrest of 90.8 % in G2/M phase of the cell cycle. Non-treated cells showed cells arrest of 50.1 %, at G1/G0 phase,16.2 % at S phase, and 28.4 % at G2/M phase of the cell cycle. (Fig-2). Thus, **HK-2** and **HK-10** might be acting like colchicine and CA-4 by binding to the colchicine binding site of the tubulin.

Table 2: Cell cycle analysis of HK-2, HK-10 and HK-13 against HCT-116 cancer cells

S. No.	**Compound name / The phase of the cell cycle**	**Control**	**Colchicine**	**HK-2**	**HK-10**	**HK-13**
1	G1/G0	50.1%	5.1%	5.6%	11.3%	**78.5%**
2	S	16.2%	4.1%	4.6%	6.8%	6.8%
3	G2/M	28.4%	**90.8%**	**88.8%**	**79.5%**	8.1%

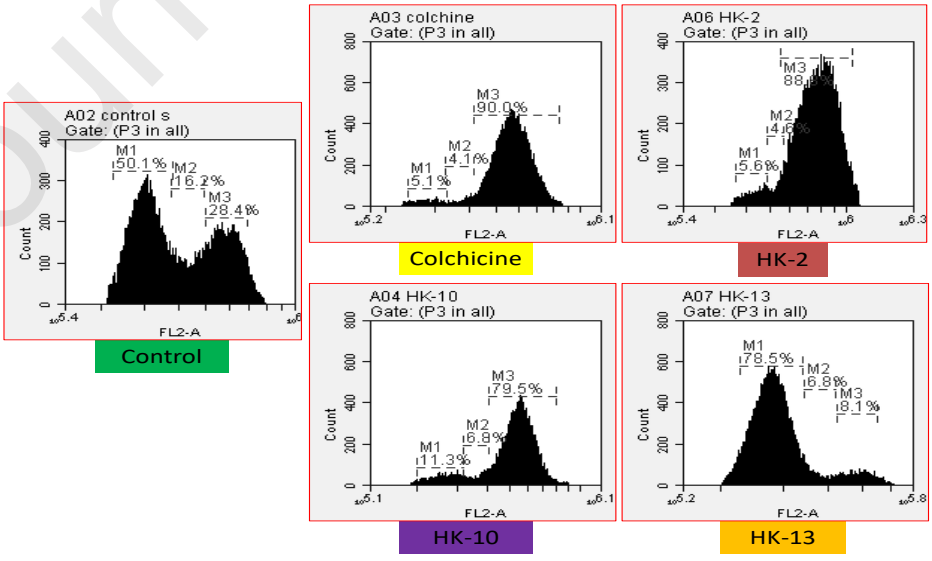


Figure 2: Cell cycle assay with propidium iodide at 12.5 µM concentration of the test compounds treated for 24h with colchicine as a positive control.

In order to understand the mechanism of cell death induced by HK-2, HK-10 and HK-13, a cytofluorimetric analysis was performed on HCT-116 cancer cells using Muse™ Annexin V and Dead Cell kit to detect the phosphatidyl serine membrane translocation. This is considered as a major hallmark of the late stage apoptosis. HCT-116 human cancer cells were treated with **HK-2, HK-10, HK-13** as test compounds and colchicine as reference at 5 µM concentrations. It has been found that HK-2, HK-10, and HK-13 showed apoptotic cell death of 48.3 %, 35.2 %, and 49.6 %, respectively as compared to 2.4 % apoptosis of the non-treated cells. Colchicine as a positive control, showed 56.4 % apoptosis after 24h treatment (Fig-3). Hence, it can be concluded that the primary mode of cell death with the tested compounds is through apoptosis.

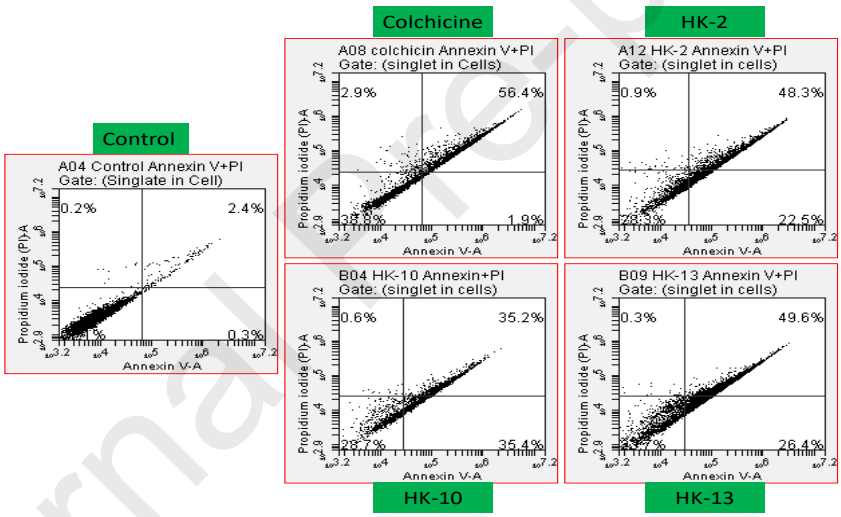


Figure-3: Quantitative analysis of apoptotic cells using AnnexinV FITC/PI double staining and flow-cytometry calculations.

in silico studies

Docking studies

From the literature search it was apparent that most of the combretastatin A-4 and colchicine derivatives bind to the colchicine binding site of the tubulin and inhibit tubulin polymerization.[44,45] **HK-2, HK-10,** and **HK-13** displayed good antiproliferative activities and in the cell cycle analysis it was found that **HK-2** and **HK-10** arrest the cell cycle at G2/M phase, typical

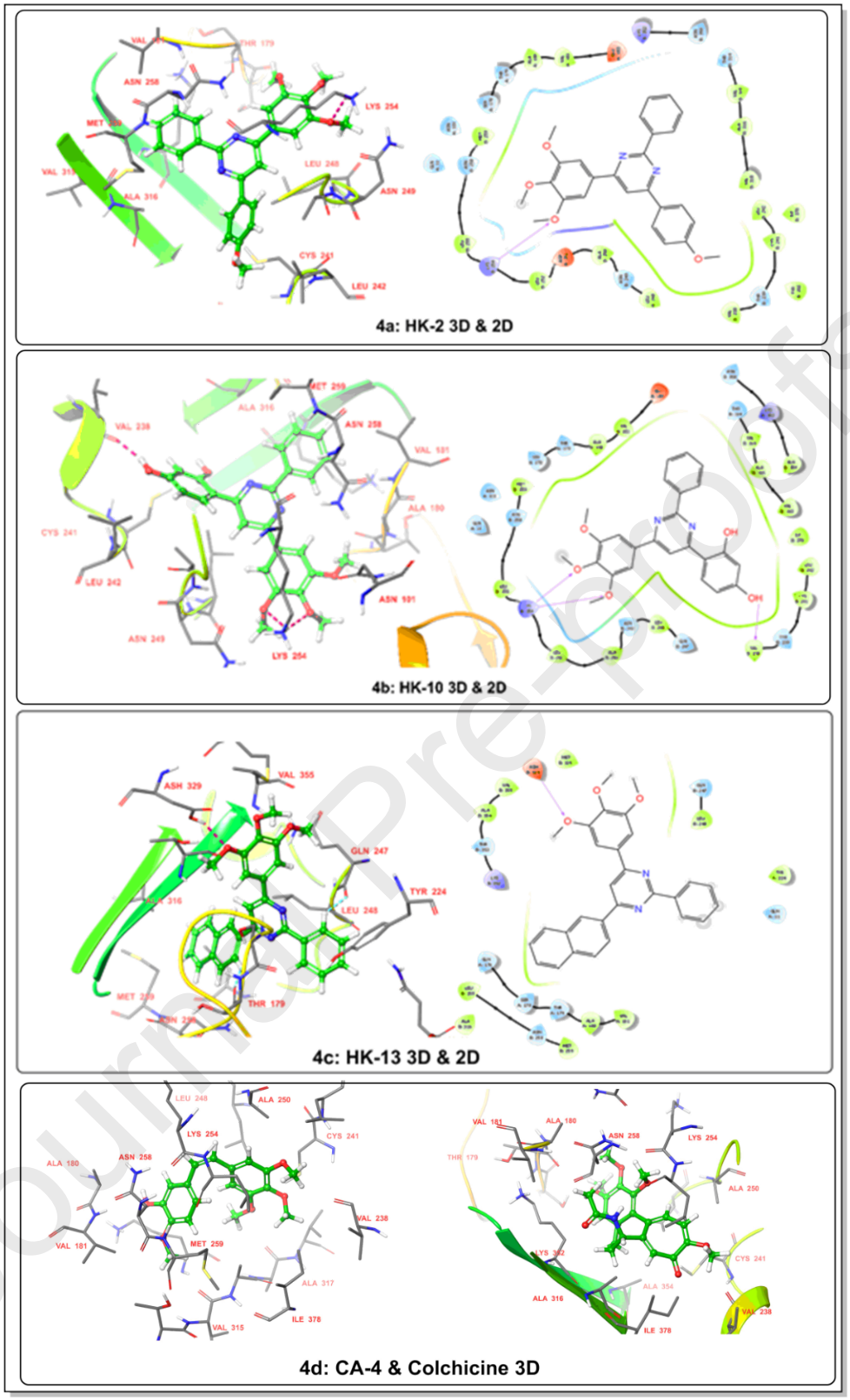


Fig:4, Docking poses of HK-2, HK-10 and HK-13 along with CA-4 and colchicine in colchicine binding site. HK-2, HK-10, HK-13, CA-4 and colchicine showed dock score of -5.82 kcal/mol, -7.85 kcal/mol, -6.15 kcal/mol, -7.95 kcal/mol and -5.5 kcal/mol, respectively.

characteristics of colchicine site binding agents. Thus, these compounds were docked into the colchicine binding site of the tubulin (PDB entry: 4O2B) to explore their interactions with the binding pocket and to investigate their binding pattern.[46] Glide (GLIDE 11.1 module of Schrödinger Suite) was used for scoring the interaction between ligands and receptor. The colchicine domain consists of colchicine binding site (main site) and its neighbouring pockets. This domain was divided into three zones i.e. zone 1, zone 2 (main site) and zone 3.[47] The docking orientations of **HK-2** and **HK-10** (Fig-4a & 4b) inside the active cavity revealed that the phenyl ring with the trimethoxy substituents fitted in the zone 2 which is delimited by Lysβ352, Asnβ350, Leuβ378, Alaβ316, Leuβ255, Lysβ254, Alaβ250 and Leuβ242, and one of the methoxy group of A ring is involved in a hydrogen bonding interaction with the distant amino group Lysβ254. In **HK-10**, the C-ring confined in zone 3 and makes hydrogen-bonding interaction with hydrogen of *p*-hydroxy group and Valβ238, while D-ring orient towards zone 1 and lined with Valα181 and Alaα180. In **HK-2**, the C-ring is also confined in zone 3 and makes hydrophobic interactions with the surrounding amino acids residues such as Thrβ239, Valβ238, which may impart better binding affinity. In **HK-13** (Fig 4c), in contrast to other colchicine binding site agents such as CA-4 (Fig 4d), the phenyl ring with trimethoxy substituents align in different direction and showed hydrogen bonding interaction with Ashβ329(a neutral Asp) and surrounded by Ashβ329, Metβ325, Leuβ248, Glnβ247. The C-ring (naphthyl ring) of HK-13 confined in zone 1 and showed hydrophobic interaction with Serα178, Thrα179, Valα181 while D-ring stayed outside the colchicine domain. Both, **HK-10** and **HK-2** showed lower dock scores of -7.25 kcal/mol and -5.82 kcal/mol, respectively as compared to reference compound colchicine with a dock score of -5.5 kcal/mol. This may be due to proper fitting of the test compounds in the active site and due to presence of additional interactions. **HK-13** with naphthyl ring (unsubstituted) as ring C showed dock score of -6.15 kcal/mol and expected to display hydrophobic interactions only. The root-mean square deviation (RMSD) of docked colchicine to the co-crystallized ligand was found to be 0.224 Å, displaying good reproducibility of the ligand binding mode.

ADME studies

The physicochemical properties of the synthesized compounds were investigated by performing ADME studies on Schrodinger's QikProp module. QikProp module utilises different parameters of Lipinski's rule of five for the analysis of drug like properties. Lipophilicity is one of the property of compounds that determine whether the molecule will cross the biological membrane or not, and for that Log P (<5) is an important physiochemical

property. **HK-2 and HK-10** displayed lipophilicity within the range of 5.71 to 6.36. Although these two compounds displayed Log P values greater than 5 but less than 6.5 which is the upper limit for druggable compounds indicating that these molecules are slightly lipophilic in nature. However, **HK-13** showed exceptionally high log P value of 7.48 and higher log P value indicates that it has some undesired characteristics such as poor aqueous solubility, rapid metabolic turnover, high plasma protein binding, and tissue accumulation. As lipophilicity plays crucial role in determining the solubility of drug candidates in biological system, and hence log S values of these compounds were also predicted. **HK-2, HK-10** and **HK-13** showed log S values of -6.98, -6.39, and -8.13, respectively as compared to standard drug CA-4 (-4.14) and colchicine (-3.07). It indicates that all the three compounds, more particularly **HK-13** would display low aqueous solubility that may compromises bioavailability during *in vivo* study. Thus, there is scope in further modification of these compounds and additional polar functionalities may be incorporated in the scaffold to increase the hydrophilicity and making these more druggable. The low values of the docking score indicate that these molecules may bind strongly with the active site. Molecular weights of these compounds were less than 500 and number of hydrogen bond donors and hydrogen bond acceptors also lies within the acceptable range (Table 2). Low value of the dipole moment, one of the factors that decide the binding of compounds with protein, of the ligands binding to the colchicine binding site is found to be better for the activity. All the three compounds showed 100 % human oral absorption and compound **HK-2 and HK-13** undergo lesser number of metabolic reactions as compared to the standard tubulin inhibitors colchicine and combretastatin A-4 (Table 3). Thus, the tested compounds satisfied the Lipinski rule of five except for the lipophilic characteristics which needs to be taken in consideration during the lead optimization.

Table 3: Drug like characteristics of compounds **HK-2, HK-10** and **HK-13** as determined by QikProp application of Schrodinger

Name	Mol. Wt.	Log P	Log S	HB donor	HB acceptor	% human oral absorption	dipole	#metb
HK-2	428.48	6.36	-6.98	0	5	100	3.806	4
HK-10	430.45	5.71	-6.39	2	5	100	2.005	5
HK-13	448.52	7.48	-8.13	0	4	100	3.854	3
Colchicine	399.44	2.704	-3.07	1	7	95.43	6.91	5
CA-4	316.35	4.045	-4.14	1	4	100	4.02	5

SAR studies

In the current study**,** 3,4,5-trimethoxyphenyl containing pyrimidine bridged combretastatin A-4 and colchicine derived compounds have been synthesized and evaluated for antiproliferative activities against MDA-MB-231, HCT-116 and HT-29 cancer cell lines. Most of the compounds displayed antiproliferative activities against these cancer cell lines in low to sub micromolar range. All the synthesized compounds composed of a trimethoxyphenyl fragment as ring A, pyrimidine moiety as ring B and a phenyl substituent on the pyrimidine as ring D. Ring C was kept as a variable fragment and selected from a group of naphthyl, thiophene, and phenyl ring optionally substituted with different types of electron withdrawing and electron releasing groups such as methyl, methoxy, amine, chloro, bromo, nitro etc. **HK-1** with para toluene group as ring C was found to be active against MDA-MB-231cell line however it showed low potency against HCT-116 and HT-29 cancer cell lines. Replacement of methyl substituent to methoxy in **HK-2** improved the activity of the compounds against all the three cell lines with IC_{50} values of 3.39 μM, 4.78 μM and 4.23 μM against MDA-MB-231, HCT-116 and HT-29, respectively. **HK-3,** with an unsubstituted phenyl ring showed almost 2 folds decrease in the activity against all the three cell lines as compared to **HK-2.** Incorporation of a hydroxy substituent at para position (HK-4) increased the activity against MDA-MB-231cells but decreased activity was observed against HCT-116 and HT-29 cell lines. Presence of electron withdrawing groups like chloro (HK-5) and bromo (HK-6) on the phenyl ring found to decrease the potency against MDA-MB-231cell line while these compounds were found to be effective against HCT-116 and HT-29 cell lines. In the series, **HK-6** was found to be the most potent against HT-29 cell line with IC_{50} value of 3.17 μM. Thiophene as ring C (HK-7), was found to be effective against MDA-MB-231cells but it showed low activity against other two cancer cell lines. Similarly, **HK-8** and **HK-9** with 2,4-dimethoxy and 3-hydroxy substituents displayed high potency against MDA-MB-231cells but showed very low activity against HCT-116 and HT-29 cell lines. **HK-10** with 2,4-dihydroxy substitution, was found effective against all the three cancer cell lines with IC_{50} values of 0.81 μM, 5.89 μM and 4.96 μM against MDA-MB-231, HCT-116 and HT-29, respectively. It was found to be the most potent compound in the current series against MDA-MB-231 cell line. **HK-11 and HK-12** with 3-nitro and 4-amino substituents, respectively showed high potency against MDA-MB-231 but these compounds were found to be less active against HCT-116 and HT-29 cell lines. Compound **HK-13** with naphthyl group as ring C, displayed significant antiproliferative activities with IC_{50} values of 3.24 μM, 4.93 μM and 4.73 μM against MDA-MB-231, HCT-116 and HT-29 cancer cell lines, respectively. In this series, many compounds were found to

show similar potency as displayed by the reference compound colchicine. Although the effect of substitution pattern on the activity, varies on different cell lines but in general it has been observed that the compounds with electron donating substituents showed higher antiproliferative activities as compared to compounds with electron withdrawing groups **HK-5** and **HK-6**. Interestingly, in this series most of the compounds were found to be more potent against MDA-MB-231 cell line as compared to other two cell lines. Thus, it can be concluded that some of the promising compounds from the current series especially **HK-2**, **HK-10** and **HK-13** can act as leads for further developments.

Pyrimidine is a versatile nucleus and indispensable constituent of many drugs and active pharmaceutical ingredients. Current series of triphenyl pyrimidines were designed as colchicine and combretastatin A-4 analogous. A total of thirteen compounds (**HK-1** to **HK-13**) were synthesized and evaluated for antiproliferative activities against MDA-MB-231, HCT-116 and HT-29 cancer cell lines. Most of the compounds showed antiproliferative activities in low to sub micromolar range. Compounds **HK-2, HK-10** and **HK-13** were found to be effective against all the three cancer cell lines. **HK-10** was found to be the most potent compound in the series with IC_{50} values of 0.81 μM against MDA-MB-231 cell line. In the series, some of the compounds displayed comparable activities to the reference colchicine. Although these compounds showed very high binding in the docking study however, low activities may be due to the high lipophilicity and their absorption & retention in the cell membrane during *in vitro* study. The promising compounds i.e. **HK-2, HK-10** and **HK-13** were subjected to cell cycle analysis and apoptosis studies to understand the mode of action of these compounds. In the cell cycle analysis, it was found that **HK-2** and **HK-10** arrest the cells in G2/M phase of the cell cycle while **HK-13** inhibit cell growth in G0/G1 phase. The ligands binding to the colchicine site arrest the cell cycle at G2/M phase and hence it was proposed that **HK-2** and **HK-10** might be binding to the colchicine site of the tubulin and killing the cells by disturbing the equilibrium between tubulin polymerization-depolymerization. It has been observed that all the three compounds inhibited cell proliferation of cancer cells by inducing apoptosis**.** In the docking studies, it was found that **HK-2 and HK-10** fit well in the colchicine binding site of the tubulin while some portion of **HK-13** stay outside the colchicine domain. Tubulin binding studies of these compounds are under process. These molecules were found to be highly lipophilic and hence further modifications in the structure can be performed to make them more potent and druggable. In conclusion, some of the triphenyl substituted pyrimidines were found to be effective against all the three cancer cell lines and these compounds can act as important leads for further studies.

Acknowledgement

A.R.D. and Vinod K. are thankful to the DST-SERB (EMR/2015/002339) for providing financial assistance. Vijay K. and NK are thankful to the UGC for the Senior and Junior Research Fellowships, respectively. SB lab is supported by a DBT Ramalingaswami Fellowship (BT/HRD/02/09/2013), DST-SERB (ECR/2016/000903) grant; RPY is supported by CSIR-Junior Research Fellowship (09/1051(0016)/2018. Authors thanks Central University of Punjab, Bathinda for providing required infrastructure. *This manuscript is dedicated to Prof. P Ramarao on his superannuation from the Central University of Punjab, Bathinda on June 30, 2020.*

Supplementary Material

Supplementary information describing detailed synthetic protocols, spectral data, biological protocols associated with this research article can be accessed in the online version.

References

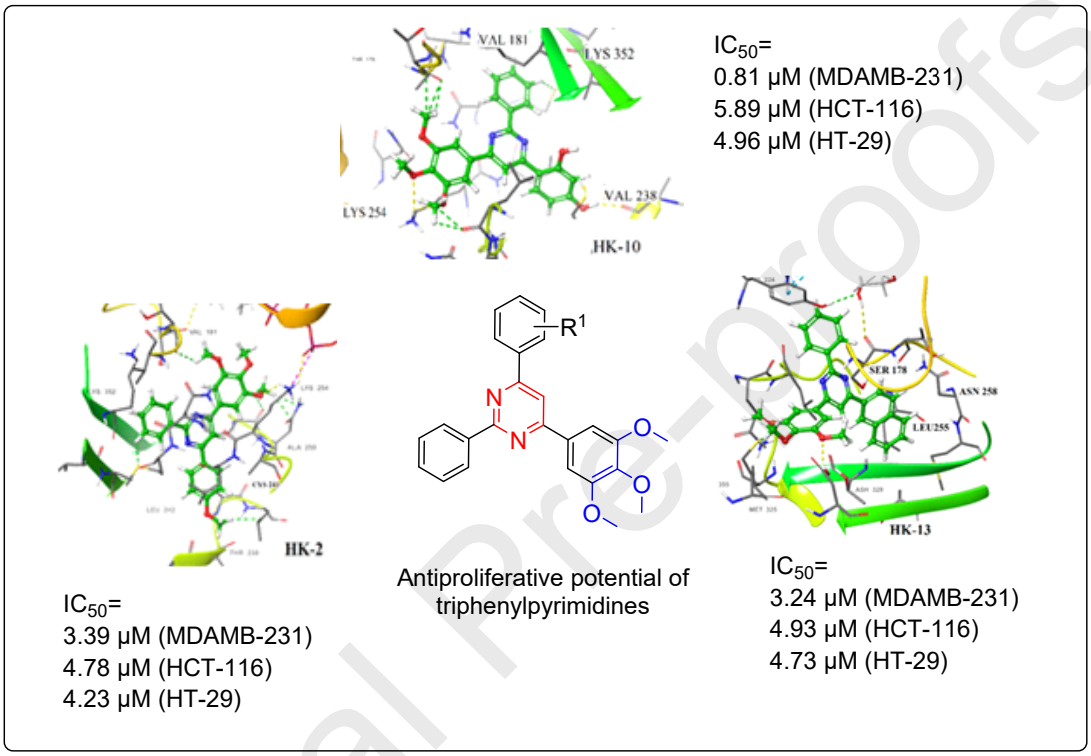
1. Vaarla K, Karnewar S, Panuganti D, Peddi S, Vedula R, Manga V, Kotamraju S. 3-(2-(5-Amino-3-aryl-1H-pyrazol-1-yl) thiazol-4-yl)-2H-chromen-2-ones as Potential Anticancer Agents: Synthesis, Anticancer Activity Evaluation and Molecular Docking Studies. *ChemistrySelect.* 2019;4(14): 4324-4330.
2. La Regina G, Coluccia A, Naccarato V, Silvestri R. Towards modern anticancer agents that interact with tubulin. *Eur J Pharm Sci.* 2019;131: 58-68.
3. Mathew A, George PS, KM JK, Vasudevan D, James FV. Transition of cancer in populations in India. *Cancer Epidemiol.* 2019;58: 111-120.
4. Dwivedi AR, Thakur A, Kumar V, Skvortsova I, Kumar V. Targeting cancer stem cells pathways for the effective treatment of cancer. *Curr. Drug Targets.* 2020;21(3): 258-278.
5. Seebacher N, Stacy A, Porter G, Merlot A. Clinical development of targeted and immune based anti-cancer therapies. *J. Exp. Clin. Cancer Res.* 2019;38(1): 156.
6. Drean A, Lord CJ, Ashworth A. PARP inhibitor combination therapy. *Crit Rev Oncol Hemat.* 2016;108: 73-85.
7. Hardin C, Shum E, Singh A, Perez-Soler R, Cheng H. Emerging treatment using tubulin inhibitors in advanced non-small cell lung cancer. *Expert Opin. Pharmacother.* 2017;18(7): 701-716.
8. Kumar B, Singh S, Skvortsova I, Kumar V. Promising targets in anti-cancer drug development: Recent updates. *Curr. Med. Chem.* 2017;24(42): 4729-4752.
9. Braguer D, Carré M, Esteve M-A. Microtubules in apoptosis induction: are they necessary? *Curr. Cancer Drug Targets.* 2007;7(8): 713-729.
10. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nat. Rev. Cancer.* 2004;4(4): 253-265.
11. Simoni D, Romagnoli R, Baruchello R, Rondanin R, Rizzi M, Pavani M, Alloatti D, Giannini G, Marcellini M, Riccioni T, Castorina M, Guglielmi M, Bucci F, Carminati P, Pisano C. Novel combretastatin analogues endowed with antitumor activity. *J. Med. Chem.* 2006;49(11): 3143-3152.

12. Zhai M, Wang L, Liu S, Wang L, Yan P, Wang J, Zhang J, Guo H, Guan Q, Bao K, Wu Y, Zhang W. Synthesis and biological evaluation of (1-aryl-1H-pyrazol-4-yl)(3, 4, 5-trimethoxyphenyl) methanone derivatives as tubulin inhibitors. *Eur. J. Med. Chem* . 2018;156: 137-147.
13. Brindisi M, Ulivieri C, Alfano G, Gemma S, Balaguer F, Khan T, Grillo A, Chemi G, Menchon G, Prota A, Olieric N, Agell D, Barasoain I, Diaz J, Nebbioso A, Conte M, Lopresti L, Magnano S, Amet R, Kinsella P, Zisterer D, Ibrahim O, Sullivan J, Morbidelli L, Spaccapelo R, Baldari C, Butini S, Novellino E, Campiani G, Altucci L, Steinmetz M, Brogi S. Structure-activity relationships, biological evaluation and structural studies of novel pyrrolonaphthoxazepines as antitumor agents. *Eur. J. Med. Chem.* 2019;162: 290-320.
14. Kumar B, Sharma P, Gupta VP, Khullar M, Singh S, Dogra N, Kumar V. Synthesis and biological evaluation of pyrimidine bridged combretastatin derivatives as potential anticancer agents and mechanistic studies. *Bioorg. Chem.* 2018;78: 130-140.
15. Li W, Sun H, Xu S, Zhu Z, Xu J. Tubulin inhibitors targeting the colchicine binding site: a perspective of privileged structures. *Future Med. Chem.* 2017;9(15): 1765-1794.
16. Kumar B, Kumar R, Skvortsova I, Kumar V. Mechanisms of tubulin binding ligands to target cancer cells: updates on their therapeutic potential and clinical trials. *Curr. Cancer Drug Targets.* 2017;17(4): 357-375.
17. Young SL, Chaplin DJ. Combretastatin A4 phosphate: background and current clinical status. *Expert Opin Investig Drugs.* 2004;13(9): 1171-1182.
18. SERIES B. Highlights from the 2016 Annual Meeting of the American Society of Clinical Oncology.
19. Rustin GJ, Galbraith SM, Anderson H, Rustin G J S, Galbraith S M, Anderson H, Stratford M, Folkes L K, Sena L, Gumbrell L, Price P M. Phase I clinical trial of weekly combretastatin A4 phosphate: clinical and pharmacokinetic results. *J. Clin. Oncol.* 2003;21(15): 2815-2822.
20. Dowlati A, Robertson K, Cooney M, Petros W P, Stratford M, Jesberger J, Rafie N, Overmoyer B, Makkar V, Stambler B, Taylor A, Waas J, Lewin J S, McCrae K R, Remick S C. A phase I pharmacokinetic and translational study of the novel vascular targeting agent combretastatin a-4 phosphate on a single-dose intravenous schedule in patients with advanced cancer. *Cancer Res.* 2002;62(12): 3408-3416.
21. Pati HN, Wicks M, Holt Jr HL, Pati H N, Wicks M, Holt H, LeBlanc R, Weisbruch P, Forrest L, Lee M. Synthesis and biological evaluation of cis-combretastatin analogs and their novel 1, 2, 3-triazole derivatives. *Heterocyclic Commun.* 2005;11(2): 117-120.
22. Kaur R, Ranjan Dwivedi A, Kumar B, Kumar V. Recent developments on 1, 2, 4-triazole nucleus in anticancer compounds: a review. Anti-Cancer Agent Med Chem *(Formerly Current Medicinal Chemistry-Anti-Cancer Agents).* 2016;16(4): 465-489.
23. Bellina F, Cauteruccio S, Monti S, Rossi R. Novel imidazole-based combretastatin A-4 analogues: evaluation of their in vitro antitumor activity and molecular modeling study of their binding to the colchicine site of tubulin. *Bioorg. Med. Chem. Lett.* 2006;16(22): 5757-5762.
24. Tsyganov DV, Konyushkin LD, Karmanova IB, et al. cis-Restricted 3-aminopyrazole analogues of combretastatins: synthesis from plant polyalkoxybenzenes and biological evaluation in the cytotoxicity and phenotypic sea urchin embryo assays. *J. Nat. Prod.* 2013;76(8): 1485-1491.
25. Zhang X, Raghavan S, Ihnat M, Hamel E, Zammiello C , Bastian A, Mooberry S, Gangjee A. The design, synthesis and biological evaluation of conformationally restricted 4-substituted-2, 6-dimethylfuro [2, 3-d] pyrimidines as multi-targeted receptor tyrosine kinase and microtubule inhibitors as potential antitumor agents. *Bioorg. Med. Chem.* 2015;23(10): 2408-2423.
26. Lu Y, Chen J, Xiao M, Li W, Miller DD. An overview of tubulin inhibitors that interact with the colchicine binding site. *Pharm. Res.* 2012;29(11): 2943-2971.
27. Hosseinzadeh Z, Ramazani A, Razzaghi-Asl N. Anti-cancer nitrogen-containing heterocyclic compounds. *Curr. Org. Chem.* 2018;22(23): 2256-2279.
28. Jain K, Arya N, Inamdar N, Auti P B, Unawane S A, Puranik H H, Sanap M S, Inamke A D, Mahale V J, Prajapati C S, Shishoo C J. The chemistry and bio-medicinal significance of pyrimidines & condensed pyrimidines. *Curr Top Med Chem.* 2016;16(28): 3133-3174.

29. Cheremnykh KP, Savelyev VA, Pokrovskii MA, Baev D S, Tolstikova T G, Pokrovskii A G Shult. Design, synthesis, cytotoxicity, and molecular modeling study of 2, 4, 6-trisubstituted pyrimidines with anthranilate ester moiety. *Med. Chem. Res.* 2019;28(4): 545-558.
30. Ragab FA, Abou-Seri SM, Abdel-Aziz SA, Alfayomy AM, Aboelmagd M. Design, synthesis and anticancer activity of new monastrol analogues bearing 1, 3, 4-oxadiazole moiety. *Eur. J. Med. Chem.* 2017;138: 140-151.
31. Park H-W, Ma Z, Zhu H, Jiang S, Robinson RC, Endow SA. Structural basis of small molecule ATPase inhibition of a human mitotic kinesin motor protein. *Sci. Rep.* 2017;7(1): 1-12.
32. Lefebvre C A, Forcellini E, Boutin S, Côté M F, Gaudreault R C, Mathieu P, Lagüe P, Paquin J F . Synthesis of novel substituted pyrimidine derivatives bearing a sulfamide group and their in vitro cancer growth inhibition activity. *Bioorg. Med. Chem. Lett.* 2017;27(2): 299-302.
33. Czudor Z, Balogh M, Bánhegyi P, Boros S, Breza N, Dobos J, Fábián M, Horváth Z, Illyés E, Markó P , Sipos A, Szántai-Kis C , Szokol B, Orf L . Novel compounds with potent CDK9 inhibitory activity for the treatment of myeloma. *Bioorg. Med. Chem. Lett.* 2018;28(4): 769-773.
34. Ma Z, Gao G, Fang K, Sun H. Development of Novel Anticancer Agents with a Scaffold of Tetrahydropyrido [4, 3-d] pyrimidine-2, 4-dione. *ACS Med. Chem. Lett.* 2019;10(2): 191-195.
35. Jin B, Tao Y, Yang H. Synthesis, Biological Evaluation and Molecular Docking of Novel Phenylpyrimidine Derivatives as Potential Anticancer Agents. *Chem. Res. **Chinise** U.* 2018;34(6): 912-917.
36. Li L, Jiang S, Li X, Liu Y, Su J, Chen J. Recent advances in trimethoxyphenyl (TMP) based tubulin inhibitors targeting the colchicine binding site. *Eur. J. Med. Chem.* 2018;151: 482-494.
37. Patel J, Malani M, Dholakiya B. Silica sulfuric acid-catalyzed Claisen-Schmidt condensation of 1, 3, 4 trisubstituted pyrrole 2, 5 dione to chalcones. *Res. Chem. Intermed.* 2012;38(9): 2371-2381.
38. Kovar SE, Fourman C, Kinstedt C, Williams B, Morris C, Choa K, Ketcha D M. Chalcones bearing a 3, 4, 5-trimethoxyphenyl motif are capable of selectively inhibiting oncogenic K-Ras signaling. *Bioorg. Med. Chem. Lett.* 2020: 127144.
39. Bandgar BP, Gawande SS, Bodade RG, Totre JV, Khobragade CN. Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory and antioxidant agents. *Bioorg. Med. Chem.* 2010;18(3): 1364-1370.
40. Katiyar SB, Bansal I, Saxena J, Chauhan P. Syntheses of 2, 4, 6-trisubstituted pyrimidine derivatives as a new class of antifilarial topoisomerase II inhibitors. *Bioorg. Med. Chem. Lett.* 2005;15(1): 47-50.
41. Kumar B, Kumar M, Dwivedi AR, Kumar V. Synthesis, Biological Evaluation and Molecular Modeling Studies of Propargyl-Containing 2, 4, 6-Trisubstituted Pyrimidine Derivatives as Potential Anti-Parkinson Agents. *Chem. Med. Chem.* 2018;13(7): 705-712.
42. Kumar B, Dwivedi AR, Sarkar B, Gupta S K, Krishnamurthy S, Mantha A K, Prakash J, Kumar V. 4, 6-Diphenylpyrimidine derivatives as dual inhibitors of monoamine oxidase and acetylcholinesterase for the treatment of Alzheimer's disease. *ACS Chem. Neurosci.* 2018;10(1): 252-265.
43. Kumar B, Kumar V, Prashar V, Saini S, Dwivedi A R, Bajaj B, Mehta D, Parkash J, Kumar V. Dipropargyl substituted diphenylpyrimidines as dual inhibitors of monoamine oxidase and acetylcholinesterase. *Eur. J. Med. Chem.* 2019;177: 221-234.
44. Ducki S. Antimitotic chalcones and related compounds as inhibitors of tubulin assembly. Anti-Cancer Agent Med Chem. 2009;9(3): 336.
45. Zhang N, Ayral-Kaloustian S, Nguyen T, Afragola J, Hernandez R, Lucas J, Gibbons J, Beyer C. Synthesis and SAR of [1, 2, 4] triazolo [1, 5-a] pyrimidines, a class of anticancer agents with a unique mechanism of tubulin inhibition. *J. Med. Chem.* 2007;50(2): 319-327.
46. Massarotti A, Coluccia A, Silvestri R, Sorba G, Brancale A. The tubulin colchicine domain: a molecular modeling perspective. *Chem. Med.Chem.* 2012;7(1): 33-42.

47. Prota AE, Danel F, Bachmann F, Bargsten K, Buey R M, Pohlmann J, Reinelt S, Lane H, Steinmetz M O. The novel microtubule-destabilizing drug BAL27862 binds to the colchicine site of tubulin with distinct effects on microtubule organization. *J. Mol. Biol.* 2014;426(8): 1848-1860.

Graphical Abstract



Highlights:

A series of triphenyl substituted pyrimidines as analogous of colchicine and combretastatin A-4 was synthesized.

Synthesized compounds were evaluated for the antiproliferative potential against MDA-MB-231, HCT-116 and HT-29 cell lines

Compounds displayed antiproliferative activity in low to sub micro molar concentration

Some of the compounds showed comparable activity as shown by standard inhibitor colchicine

Lead compounds showed cell arrest at G2/M phase and cell death induced through apoptosis