



Synthesis and biological evaluation of new 2,4,6-trisubstituted pyrimidines and their *N*-alkyl derivatives

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ABSTRACT

A series of new 2,4,6-trisubstituted pyrimidines and their *N*-alkyl bromide derivatives were prepared based upon methoxy substituted azachalcones as the starting materials. All newly synthesized compounds were screened for their anti-proliferative, cytotoxic, antibacterial activities and DNA/protein binding affinity. *In vitro* cell proliferation inhibitory and cell cytotoxic effects of 2,4,6-trisubstituted pyrimidines (1–9) and their *N*-alkyl bromide derivatives (2a-c, 3a-c, 5a-c, 6a-c, 8a-c, 9a-c) were obtained with the help of the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) cell proliferation, LDH cytotoxicity detection, and microdilution assays. The antimicrobial activity for these compounds was also evaluated following the European Pharmacopoeia 8.0 protocol. The interactions of these compounds with DNA or bovine serum albumin were investigated by the spectrophotometric titration method. When the cytotoxic analysis and anticancer properties of the compounds were examined, most of the compounds significantly exhibited an anti-proliferative potency on cancer cells (IC₅₀ ~ 2–10 µg/mL) and caused a cytotoxic effect as low as control drugs, 5-fluorouracil, and cisplatin (~7–15%). Because the compound-DNA adducts are hyperchromic or hypochromic, they caused variations in their spectra. This situation shows they can be linked to DNA by the groove binding mode at a binding constant range of 2.0×10^4 and $2.4 \times 10^5 \text{ M}^{-1}$. The antimicrobial screening results revealed that our new compounds for some human Gram(+) and Gram(-) pathogen bacteria showed remarkable activity with MIC values between < 7.81 and 125 µg/mL. Overall, incorporation of alkyl chain to pyrimidines in the generation of *N*-alkyl bromides has resulted in showing differences in DNA/protein binding affinity, along with anti-proliferative and cytotoxic activity in favor of new compounds.

1. Introduction

Pyrimidine and derivatives are important subunits and they have been found in a variety of natural products that are important for the synthesis of pharmaceutical and agrochemical compounds. Pyrimidine ring system has extensive occurrence in nature, including the alloxan, alkaloids, nucleic bases, thiamine (vitaminB1), and numerous pharmacophores [1–3]. They are also found in many synthetic compounds such as barbiturates and the HIV drug, zidovudine [4]. Pyrimidine derivatives occupy an important place in medicinal chemistry with the exhibited antimicrobial [5,6], anticancer [4,5,7,8], antifungal [4], analgesic [9], antiviral [5], anticonvulsant [10], anti-inflammatory [5], antitubercular [6], antifungal [4], antileishmanial [11] and

antimalarial activities [12–14].

Some of the of pyrimidine containing drugs, sulfadiazine, sulfamerazine, sulfamethazine, and trimethoprim (antimicrobial), iodoxuridine, trifluoridine and zidovudine show that antiviral, 5-fluorouracil, florafur and piritrexim, isetionate (anticancer), flucytosine (antifungal) and pyrimethamine (antimalarial) have been used clinically [15–17] (Fig. 1).

Moreover, different conjugated pyrimidines have luminescence properties and therefore they have been used in organic light emitting devices (OLED) and molecular wires. They also perform as ligands in transition metal complexes sometimes forming supramolecular assemblies. Due to the great importance of the pyrimidine moieties, a number of efficient methods have been reported in literature for their synthesis

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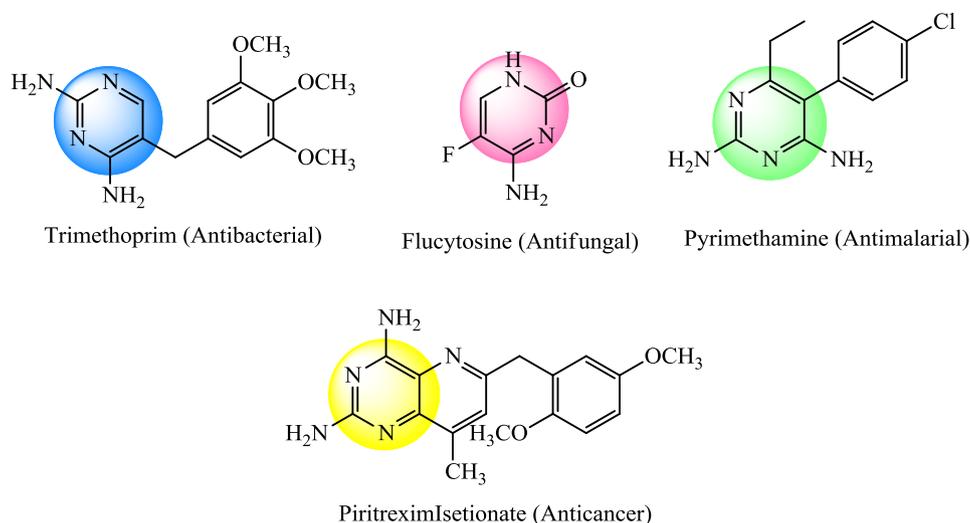


Fig. 1. Structure of some drugs bearing pyrimidine core.

[3,5,7,11–14,18–27].

Chalcones are the most used and very attractive bioactive starting materials for the synthesis of substituted pyrimidines. Substituted chalcones and guanidine salts can easily react in basic conditions according to 1,4-Michael addition and give 2,4,6-trisubstituted pyrimidines [5,19,20,22,11–14,25,28]. In recent years, *N*-alkyl substituted forms of heterocyclic compounds including azachalcone, azaflavone, azaflavanone, pyridine, carbazole, indole, and phenothiazine units have acquired much attention for their comprehensive inclusion in chemistry, biology, and materials science [29–34]. The salt forms of these compounds have an especially important role in agriculture, the food processing industry, clinics and medicinal chemistry due to their high biological activity [30–35] because of the cation form of the synthesised organic compounds.

Recently, there has been considerable interest for the synthesis of substituted pyrimidine due to their broad biological activities. Therefore, there still is a need to synthesize a new series of substituted pyrimidine and their *N*-alkylpyridinium salts to explore the pharmaceutical relevance. Hence, the aim of our research was to synthesize a series of 2,4,6-trisubstituted pyrimidines (1–9) and their 5, 10 and 15 carbon containing *N*-alkyl bromide derivatives (2a-c, 3a-c, 5a-c, 6a-c, 8a-c, 9a-c) and to screen for their antibacterial, DNA/protein binding affinity and anticancer activities [4,5,15–17,20,11–14,24,25].

2. Results and discussion

2.1. Chemistry

The known sequential synthesis of the 2,4,6-trisubstituted pyrimidines (1–9) and their *N*-alkylpyridinium bromides (2a-c, 3a-c, 5a-c, 6a-c, 8a-c, 9a-c) are depicted in Scheme 1.

In the first part of the work, the designed pyrimidine compounds were synthesized by the reaction which goes on either by the 1,2-addition and/or 1,4-addition (Michael addition) of guanidine to the α , β -unsaturated carbonyl part of azachalcone, traced by cyclization to give the corresponding 2-amino-4,6-disubstituted pyrimidines 1–9 [11–14,20,24,25]. In the second step, the target *N*-alkylpyridinium bromides (2a-c, 3a-c, 5a-c, 6a-c, 8a-c, 9a-c) were obtained by the bimolecular substitution reaction between pyridinyl nitrogen of pyrimidine and alkyl bromide [30–35]. The purity of all the synthesized compounds was checked by TLC and all synthetic compounds were purified by crystallization or column chromatography and their structures were identified by spectroscopic methods.

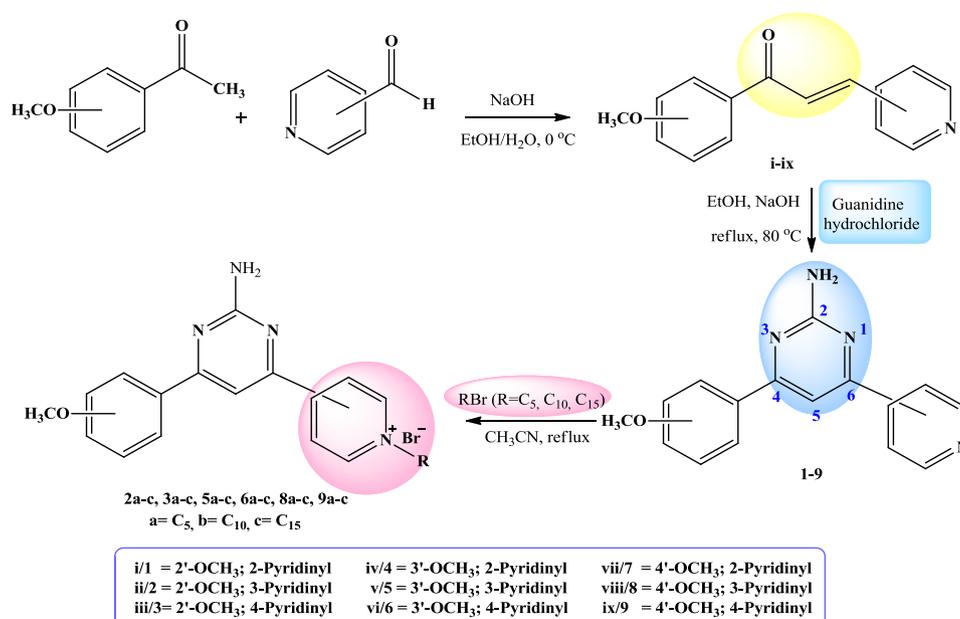
FT-IR spectra of target compounds showed two bands in the regions

of 1661–1605 cm^{-1} and 1368–1356 cm^{-1} which are characteristic for pyrimidine ring [20]. These bands emerged after the intermolecular cyclization between chalcone and guanidine and own to $\text{C}=\text{N}$ vibrations. The disappearance of the carbonyl group absorption band at about 1680 cm^{-1} also supported the formation of a pyrimidine structure. FT-IR spectra of pyrimidines and *N*-alkylated pyrimidines gave bands between 3457 and 3077 cm^{-1} and 3495–3179 cm^{-1} , which provided more evidence for the free and H-bonded NH_2 absorptions, respectively [20].

The ^1H NMR spectra of target compounds exhibited further support for the 2-aminopyrimidine cycle, since they indicated a broad singlet for NH_2 at δ 5.2–5.4 ppm in pyrimidines and δ 5.4–6.0 ppm in alkylated pyrimidines. The reason for the high field chemical shift of alkylated compounds is their ionic form and ^1H and ^{13}C NMR spectra of all synthetic compound revealed the characteristic peaks at 7.5–8.2 ppm (^1H) and 103.6–109.9 ppm (^{13}C) for the H-5 and C-5 of pyrimidine ring [20]. All other carbon peaks in the ^{13}C NMR spectra for the synthetic pyrimidine compounds were found in the range of 111.4–167.9 ppm.

LC-MS/MS spectra of all synthetic compounds were characterized by medium or low intensity molecular ions peaks at the appropriate m/z values. The main molecular ion peaks of compounds 1–9 showed $[\text{M} + \text{Na}]^+$, $[\text{M} + 1]^+$ or $[\text{methoxyphenyl-1}]^+$ and alkylated pyrimidines showed $[\text{M} - ^{79}\text{Br}/^{81}\text{Br}]^+$ fragments as the base peaks in the mass spectra.

In the second part of the study, we synthesized *N*-alkylpyridinium bromides containing 5, 10 and 15 carbon chains. As described above, the synthesis of these compounds was carried out by $\text{S}_{\text{N}}2$ reaction and the linking of the alkyl chain to the pyridine nitrogen was confirmed by spectroscopic results. The observed triplet peak at about 4.7–5.1 ppm in ^1H NMR and the carbon peak at about 61.8–62.8 ppm in ^{13}C NMR spectra of *N*-alkylpyridinium bromides were typical to $\text{N}-\text{CH}_2-$ chemical shifts and were evidence for the alkylation of compounds 1–9 due to the strong pK_{b} value of pyridine (8.77) versus the pyrimidine (11.3) [16]. Compared to pyridine, *N*-alkylation of pyrimidine is more difficult [16]. Also, the electron density of the free amino group is decreasing by the mesomeric effect and in this case, it behaves as a poor nucleophile. After alkylation, the chemical shifts of pyridine protons shifted to a low field. For example, when the chemical shifts of compounds 2 and 2c in ^1H NMR spectra were compared, it was clearly seen there was almost no change in chemical shifts of the pyrimidine and methoxyphenyl protons, while the pyridine protons of 2c shifted to low field (δ (2/2c, ppm) = 7.7/7.9 (H-5), 7.1/7.1 (H-3'), 7.5/7.5 (H-4'), 7.1/7.1 (H-5'), 7.9/7.9 (H-6'), 9.3/9.6 (H-2''), 8.7/9.9 (H-4''), 7.4/8.3



Scheme 1. Sequential synthesis of 2,4,6-trisubstituted pyrimidines (1–9) and their *N*-alkylpyridinium bromides (2a-c, 3a-c, 5a-c, 6a-c, 8a-c, 9a-c).

(H-5''), and 8.4/9.0 (H-6'')). In addition to all the above, the synthesis of **1a-c**, **4a-c** and **7a-c** couldn't be obtained because of the steric hindrance of pyridine nitrogen of pyrimidines **1**, **4** and **7** even under different reaction conditions. Example studies are available in literature about this [28,29].

All the newly synthesized compounds were characterized by spectroscopic data including ¹H, ¹³C, APT, COSY, ACD-NMR, FT-IR, LC-MS/MS, and elemental analysis. All data obtained from the spectral analyses for all compounds were in full agreement with the proposed structures.

N-alkylation of target 2-pyridinyl compounds (**1a-c**, **4a-c** and **7a-c**) were not obtained. This could be the steric hindrance for the pyridine nitrogen at two positions as described in the literature [32,33].

2.2. Biological evaluation

2.2.1. Evaluation of anticancer properties of molecules

Despite the intensive work on effective cancer treatment, 11 million people still have cancer in the world, and 7 million people lose their lives due to cancer. Another important issue is that 3 out of every 5 cancers occur in countries with lower and middle incomes and therefore they are faced with a much higher degree of disease and economic burden. For this reason, intensive studies could be designed to synthesise cheap, easily obtainable and effective chemical molecules for the treatment of cancer in order to prevent deaths. Thus, we evaluated the anticancer effects of the new synthesised 2,4,6-trisubstituted pyrimidines (1–9) and their 5, 10 and 15 carbon chain containing *N*-alkyl bromide derivatives (2a-c, 3a-c, 5a-c, 6a-c, 8a-c, 9a-c), using the MTT protocol. IC₅₀ inhibition values generally used for the inhibition studies and GI₅₀, TGI and LC₅₀ parameters were determined as suggested by NCI from spectrophotometric data obtained from the MTT assay using cisplatin and 5FU as control anticancer drugs. It was seen that compounds 1–9 were not sufficiently anti-proliferative on lung cancer cell line A549 when compared with the control group, even at a high concentration of 500 µg/mL (Table 1).

Nevertheless, pyrimidines and their alkylated derivatives showed very strong anticancer activity on C6 cell line (TGI 3.64–21.31 µg/mL and IC₅₀ 5.51–27.46 µg/mL for pyrimidines), (TGI 1.01–12.16 µg/mL and IC₅₀ 1.01–17.48 µg/mL for alkyl derivatives) except compound **7** (Table 2).

Many of the compounds also showed a very high anti-proliferative effect on HeLa cell line, except compounds **1**, **3**, **7**, and **9** (TGI

1.01–42.06 µg/mL and IC₅₀ 1.0–47.22 µg/mL for alkyl derivatives) (TGI 5.80–18.07 µg/mL and IC₅₀ 8.86–34.64 µg/mL for pyrimidines). According to the HT29 cell line investigations, synthesized compounds except **1**, **3**, and **7** exhibited much better anti-proliferative performance than the positive controls (TGI value 1.19–14.33 µg/mL and IC₅₀ value 1.14–11.71 µg/mL for *N*-alkyl bromide derivatives) (TGI value 8.01–182.50 µg/mL and IC₅₀ value 6.57–53.02 µg/mL for 2,4,6-trisubstituted pyrimidines) except compounds **1**, **3**, and **7** (Table 2). The compounds **2**, **9** (IC₅₀ 71.22 and 22.46 µg/mL, respectively) and **2b-c**, **3a-c**, **5a-c**, **6b-c**, **8b-c**, **9a-c** (IC₅₀ 3.46–68.10 µg/mL), which were effective in the MCF7 cell line, showed considerably high anticancer activities as shown in Table 1. The alkyl derivatives **2b-c**, **3b-c**, **5a-c**, **6b-c**, **8b-c**, and **9b-c** further exhibited very strong anticancer properties (IC₅₀ 5.57–39.48 µg/mL) on the A549 cancer cell line. It can be easily seen that the compounds **2**, **6**, and **8** (IC₅₀ 22.50, 7.63, and 8.46 µg/mL, respectively) and **2b-c**, **3b-c**, **5a-c**, **6b-c**, **8b-c**, and **9b-c** (IC₅₀ 6.01–35.60 µg/mL) indicated a remarkable anti-proliferative effect against hepatocellular carcinoma cell line Hep2B. Anti-proliferative effects of compounds **2**, **4**, **5**, **6**, and **8** (IC₅₀ value 11.58–35.41 µg/mL) and **2a-c**, **3a-c**, **5a-c**, **6b-c**, **8a-c**, and **9a-c** (IC₅₀ 1.44–48.76 µg/mL) towards the FL cell line had similar behavior to other cells. While the molecules that were found as anticancer effective according to the TGI and IC₅₀ values above, they were also examined in terms of LC₅₀ values suggested by NCI and the following results were obtained. The compounds **8** (LC₅₀ 222.34 µg/mL) for cervical cancer, **3** for glioma cancer (LC₅₀ 305.89 µg/mL), **2**, **4**, **5**, **6**, **8**, **9**, **2a**, **3a**, **6a**, **8a**, and **9a** for colon cancer (LC₅₀ values ranging from 125.09 µg/mL to > 500 µg/mL), **2**, **9**, **2b-c**, **3a**, **5a-b**, **8a-c**, and **9a** for breast cancer (LC₅₀ 120.20 µg/mL – > 500 µg/mL), **2b**, **5a-b**, and **8b** (LC₅₀ 449.23 µg/mL – > 500 µg/mL) for lung cancer and **2**, **8**, **2b-c**, **5a-b**, **8b-c**, and **9c** (LC₅₀ 156.59 µg/mL – > 500 µg/mL) for hepatocellular carcinoma are highly recommended for further pharmacological studies since they exceeded very high LC₅₀ values. It showed that high LC₅₀ values of compounds **2**, **4**, **5**, **6**, **8**, **9**, **3a**, **5b**, **8a-b**, and **9a** were not too lethal on normal cell line when LC₅₀ values of test molecules were examined on normal cell line (FL) (Tables 3 and 4).

For this reason, these molecules are pharmacologically valuable and may be candidates for phase studies. When other effective molecules are examined, they need to be reformulated because they have very low LC₅₀ values and they also have low GI₅₀ values. High lethal concentration values indicate that the cytotoxic effects of the test

Table 1

GI₅₀, TGI, LC₅₀ and IC₅₀ values for 1–9 and 2a-c, 3a-c, 5a-c, 6a-c, 8a-c, 9a-c against MCF7, A549, and Hep3B.

Compound (µg/mL)	MCF7				A549				Hep3B			
	GI ₅₀	TGI	LC ₅₀	IC ₅₀	GI ₅₀	TGI	LC ₅₀	IC ₅₀	GI ₅₀	TGI	LC ₅₀	IC ₅₀
1	2.64	> 500	> 500	> 500	15.78	> 500	> 500	> 500	4.40	> 500	> 500	> 500
2	2.52	78.95	> 500	71.22	5.92	> 500	> 500	> 500	3.20	23.08	> 500	22.50
3	1.65	466.56	> 500	334.35	136.68	> 500	> 500	> 500	3.44	> 500	> 500	> 500
4	1.65	122.83	> 500	98.99	213.49	> 500	> 500	> 500	2.68	101.04	> 500	92.82
5	1.80	> 500	> 500	> 500	21.34	> 500	> 500	> 500	3.68	119.39	> 500	111.37
6	1.46	440.76	> 500	307.11	130.24	> 500	> 500	> 500	2.56	7.73	84.87	7.63
7	1.38	> 500	> 500	> 500	317.05	> 500	> 500	> 500	3.46	> 500	> 500	> 500
8	1.33	> 500	> 500	> 500	14.99	> 500	> 500	> 500	1.71	8.73	> 500	8.46
9	1.83	24.90	> 500	22.46	26.15	> 500	> 500	> 500	2.24	125.67	> 500	112.85
2a	4.51	> 500	> 500	> 500	7.25	> 500	> 500	> 500	4.75	127.20	> 500	121.17
2b	2.65	9.94	222.59	9.67	5.68	50.67	> 500	39.48	3.44	16.98	> 500	16.68
2c	2.67	8.73	120.20	8.55	2.91	8.21	63.64	7.20	3.44	12.89	197.47	12.73
3a	2.81	70.30	> 500	63.31	31.94	> 500	> 500	> 500	6.77	> 500	> 500	> 500
3b	1.83	4.56	45.07	4.46	3.11	9.05	72.08	7.96	2.22	6.09	60.42	6.01
3c	2.55	6.81	50.81	6.71	2.81	7.07	40.68	6.40	3.24	9.40	71.36	9.32
5a	2.57	10.03	280.29	9.75	4.26	19.61	449.23	16.11	3.77	14.66	227.85	14.49
5b	2.68	10.75	304.59	10.47	5.35	42.00	> 500	33.21	4.13	26.74	> 500	26.23
5c	2.23	5.61	41.01	5.52	2.60	6.13	31.13	5.57	2.72	7.85	69.07	7.77
6a	1.97	419.32	> 500	319.02	32.41	> 500	> 500	> 500	3.69	> 500	> 500	> 500
6b	1.56	3.54	35.75	3.46	2.77	6.83	37.22	6.18	2.51	6.65	49.06	6.58
6c	2.32	5.74	37.58	5.65	2.70	6.40	32.01	5.82	3.10	8.10	47.86	8.04
8a	3.51	43.23	> 500	40.29	40.75	> 500	> 500	> 500	4.89	> 500	> 500	> 500
8b	2.57	9.72	238.47	9.45	4.09	31.70	> 500	23.97	4.36	36.42	> 500	35.60
8c	2.73	9.03	123.53	8.84	3.14	8.40	52.64	7.51	3.64	13.89	212.63	13.72
9a	2.97	74.75	> 500	68.10	33.24	> 500	> 500	> 500	4.90	171.61	> 500	162.50
9b	2.09	5.54	52.70	5.43	3.54	10.44	77.38	9.43	2.72	8.27	86.81	8.17
9c	2.08	5.81	67.66	5.68	3.29	9.35	66.32	8.39	2.63	9.11	156.59	8.98

substances are less and this condition is desirable. Low GI₅₀ and TGI values indicate that the cytotoxic effects of the test substances are greater, and again this is a desirable condition. When the anti-proliferative properties of *N*-alkyl bromide derivatives were examined, the anti-proliferative properties of **b** and **c** series were prominently increased except series **a**. This increase is nevertheless important. It does

bring undesirable cytotoxic characters, because when GI₅₀ and TGI values are examined (Tables 5 and 6), the effect of these molecules is extraordinarily high (GI₅₀ and TGI values 1–2 µg/mL) in any anticancer agent used in contemporary cancer clinics.

Due to this particular situation, we would like to see the full pharmacological potential of these group molecules on the C6 and HeLa cell

Table 2

GI₅₀, TGI, LC₅₀ and IC₅₀ values for 1–9 and 2a-c, 3a-c, 5a-c, 6a-c, 8a-c, 9a-c against C6, HeLa, and HT29.

Compound (µg/mL)	C6				HeLa				HT29			
	GI ₅₀	TGI	LC ₅₀	IC ₅₀	GI ₅₀	TGI	LC ₅₀	IC ₅₀	GI ₅₀	TGI	LC ₅₀	IC ₅₀
1	3.98	12.51	101.72	19.85	> 500	> 500	> 500	> 500	1.43	> 500	> 500	413.34
2	3.60	8.25	32.34	11.96	8.67	18.20	49.20	21.86	2.12	8.01	320.22	6.57
3	1.53	4.78	305.89	10.86	> 500	> 500	> 500	> 500	1.30	> 500	> 500	79.78
4	1.73	3.64	20.95	5.51	2.47	5.80	30.49	8.86	1.32	11.49	> 500	6.70
5	3.29	8.13	40.02	12.20	6.29	18.21	97.23	27.72	1.73	24.09	> 500	13.74
6	3.21	8.19	44.11	12.66	7.23	17.72	65.20	23.40	1.99	9.18	> 500	7.18
7	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	1.73	427.61	> 500	90.31
8	5.83	16.53	86.64	24.65	4.71	18.07	222.34	34.64	1.68	13.98	> 500	9.11
9	7.75	21.31	96.53	27.46	> 500	> 500	> 500	> 500	1.72	182.50	> 500	53.02
2a	2.30	4.86	20.14	7.01	5.91	13.30	43.28	17.63	2.53	9.47	233.38	8.07
2b	1.00	1.02	1.66	1.06	1.00	1.01	1.09	1.00	1.38	2.34	9.41	2.21
2c	1.00	1.02	1.62	1.06	1.00	1.01	1.13	1.00	1.02	1.19	3.50	1.14
3a	1.60	3.00	13.11	4.26	10.40	21.03	52.55	24.23	3.23	11.87	183.98	10.48
3b	1.00	1.01	1.22	1.01	1.00	1.01	1.24	1.01	1.04	1.27	4.49	1.20
3c	1.00	1.01	1.07	1.00	1.00	1.01	1.50	1.04	1.57	2.81	10.67	2.69
5a	1.00	1.01	1.52	1.03	1.00	1.02	1.84	1.05	1.93	3.85	15.89	3.62
5b	1.00	1.01	1.44	1.02	1.00	1.03	1.63	1.04	2.31	5.21	25.70	4.81
5c	1.00	1.01	1.32	1.01	1.00	1.02	1.86	1.07	2.46	5.47	24.68	5.11
6a	4.92	12.16	50.13	17.48	21.13	42.06	97.81	47.22	2.76	14.33	> 500	11.71
6b	1.00	1.03	1.78	1.08	1.00	1.02	1.60	1.04	1.90	4.01	20.13	3.65
6c	1.00	1.02	1.54	1.04	1.00	1.01	1.42	1.02	1.42	2.47	10.25	2.24
8a	2.73	5.88	22.78	8.42	8.48	18.90	56.86	24.22	2.30	7.43	125.09	6.38
8b	1.00	1.01	1.21	1.01	1.00	1.01	1.44	1.03	1.95	4.44	27.98	4.02
8c	1.00	1.01	1.34	1.02	1.00	1.01	1.38	1.02	2.53	6.41	41.42	5.82
9a	3.94	9.00	33.82	12.94	6.01	13.72	45.77	19.06	2.42	9.69	340.78	8.05
9b	1.00	1.01	1.35	1.02	1.00	1.01	1.12	1.00	2.15	4.75	23.77	4.37
9c	1.00	1.02	1.62	1.06	1.00	1.02	1.40	1.02	2.77	10.96	276.91	9.31

Table 3
GI₅₀, TGI, LC₅₀ and IC₅₀ values for 1–9 and 2a–c, 3a–c, 5a–c, 6a–c, 8a–c, 9a–c against FL.

Compound (µg/mL)	FL				Compounds (µg/mL)	FL			
	GI ₅₀	TGI	LC ₅₀	IC ₅₀		GI ₅₀	TGI	LC ₅₀	IC ₅₀
1	5.83	> 500	> 500	> 500	5a	2.88	7.86	55.29	7.80
2	3.86	23.36	> 500	22.96	5b	3.08	9.99	110.52	9.90
3	3.01	115.61	> 500	108.91	5c	1.06	1.45	10.94	1.44
4	3.38	32.27	> 500	31.34	6a	3.85	> 500	> 500	> 500
5	3.14	13.66	392.98	13.48	6b	1.25	2.21	16.36	2.19
6	2.83	11.74	343.44	11.58	6c	1.55	3.23	23.52	3.20
7	8.29	> 500	> 500	> 500	8a	4.48	23.30	> 500	22.98
8	3.40	36.57	> 500	35.41	8b	3.52	13.02	187.22	12.88
9	4.98	358.00	> 500	331.06	8c	1.79	4.03	27.74	4.00
2a	4.80	35.43	> 500	34.66	9a	4.70	49.89	> 500	48.76
2b	2.90	8.93	90.53	8.84	9b	1.39	2.66	17.86	2.64
2c	1.68	3.71	27.88	3.68	9c	1.85	4.22	29.35	4.19
3a	4.32	44.45	> 500	43.37					
3b	1.09	1.58	11.75	1.57					
3c	1.36	2.64	21.31	2.62					

Table 4
IC₅₀ (µg/mL) of positive controls in cell lines.

	HeLa	HT29	A549	MCF7	C6	Hep3B	FL
Cisplatin	50.29	40.39	60.49	63.79	33.08	48.69	52.79
5FU	61.59	65.19	69.79	74.19	54.30	62.89	59.09

lines by conducting an ELISA BrdU assay, which is a more sensitive measurement, in the next study. We did not have a positive control that could help us with the nanomolar dose we used, so we had to evaluate the effect in and of itself. When the ELISA BrdU and the MTT test results described below were evaluated together, it was seen that the a, b and c series compounds were suitable for further testing in both types of cancer. When the IC₅₀ data of the test results were examined, except 6a and 9a, a series compounds exhibited anticancer activity at the microgram level similar to 5-fluorouracil (5FU) and cisplatin, which were control anticancer molecules, on C6 and HeLa cells (Tables 5 and 6). When the GI₅₀ values as given in Tables 5 and 6 were examined, b and c series compounds showed more growth inhibition on C6 and HeLa cell lines than cisplatin and 5FU at the nanogram level. According to the data given in Tables 7 and 8, the c series compounds were more anti-proliferative than others.

In general, all compounds of the c series and only 9b of the b series exposed exceptional anti-proliferative properties. ELISA BrdU measurement results also showed us that the test compounds were not highly anti-proliferative to the normal cell line FL (GI₅₀ values about 1000 ng/mL, TGI values about 3000 ng/mL, LC₅₀ values about 7000 ng/mL) (Table 6). This situation showed that the test compounds were cancer specific. The cytotoxic effects on the cells as well as the anticancer activities of the tested compounds were determined by the membrane damage measurement technique. When we evaluated the LDH activity measurement results, it was found that the above-

Table 5
GI₅₀, TGI, LC₅₀ and IC₅₀ values for 2a, 3a, 5a, 6a, 8a, 9a against C6, HeLa, and FL^a.

Compound (µg/mL)	GI ₅₀			TGI			LC ₅₀			IC ₅₀		
	C6	HeLa	FL	C6	HeLa	FL	C6	HeLa	FL	C6	HeLa	FL
2a	17.5	37.9	5.45	43.2	183.1	33.66	141.7	> 500	> 500	58.9	179.1	26.26
3a	15.1	250.2	4.07	51.1	> 500	74.49	296.3	> 500	> 500	108.2	> 500	43.17
5a	9.3	15.2	3.22	18.5	29.6	8.56	46.02	67.5	51.80	23.5	35.53	7.62
6a	114.1	> 500	5.66	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500
8a	25.6	72.8	4.14	71.2	> 500	19.61	> 500	> 500	> 500	121.9	192.3	15.62
9a	32.9	104.3	2.72	275.2	> 500	17.14	> 500	> 500	> 500	> 500	> 500	11.39

^a These parameters were determined by ELISA BrdU Assay.

mentioned anticancer compounds 5, 8, 2a, 3a, and 6a for C6 cells, 3, 4, 7, 5b, 8b, and 9b for HeLa cells, 2a–b, 3b, 6a, and 9a–b for A549 cells, 6a–b, 8a–b, and 9a for the Hep3B cells, 2a–b, 3a, 5a–b, 8b, and 9a–b for MCF7 cells, 2a, 3a, and 8b for HT29 cells, and 2a, 5a, and 8b for FL cells caused membrane damage in the range of 5.57% - 18.37% at their IC₅₀ concentration (Tables 7 and 9). These values are very close to the cytotoxicity values % caused by our positive controls (5FU and Cisplatin) (Table 8). The results of the pharmacological evaluation of the new molecules described above on working cell lines largely meet the NCI criteria.

2.2.2. Morphological changes that molecules cause on cells

Changes in cell morphology was found with the help of phase-contrast microscopy as a result of incubation of 2,4,6-trisubstituted pyrimidines and their N-alkyl bromide derivatives after 24 h at similar concentration ratios used in the above tests. The findings of this study are in accord with the morphological changes observed in apoptotic cells such as cytoplasmic extensions of bubble, pinhole, and pocket formation detected on phase-contrast microscopy (Figs. 2 and 3). At the end of the treatment with the test substances, cellular debris originating from some necrotic cells and some apoptotic cells were also found. Moreover, the effect of newly synthesized compounds at low concentrations on cells were so small that it didn't change the normal appearance of the cells. However, cells tested at high concentrations did not maintain normal morphology (Figs. 2 and 3).

2.2.3. Evaluation of antibacterial effects of molecules

The resistance of bacteria that cause non-healing infections to antibiotics is a major global health problem nowadays [36]. For this reason, there is a need to develop and discover new antimicrobial agents that are more effective against antibiotic-resistant bacteria. The study of the antibacterial effects of some of the new compounds synthesized by our research group on resistant bacteria that cause disease

Table 6
GI₅₀, TGI, LC₅₀ and IC₅₀ values for **2b-c**, **3b-c**, **5b-c**, **6b-c**, **8b-c**, **9b-c** against C6, HeLa, and FL^a.

Compound (ng/mL)	GI ₅₀			TGI			LC ₅₀			IC ₅₀		
	C6	HeLa	FL	C6	HeLa	FL	C6	HeLa	FL	C6	HeLa	FL
2b	247	289	1602	748	860	3586	2822	3158	8766	1187	1469	3262
3b	373	37	633	1002	154	1704	3177	1111	5342	1386	356	1504
5b	922	943	1505	1971	2075	3861	4583	4996	11,179	2364	2614	3400
6b	156	213	1311	511	676	2738	2215	2746	6167	936	1254	2568
8b	231	810	2438	696	1844	4879	2620	4641	10,387	1144	2402	4614
9b	85	83	1364	318	309	2836	1732	1683	6354	662	644	2661
2c	1	1	1798	1	4	3610	4	61	7730	2	12	3421
3c	2	1	1105	6	3	2423	82	44	5802	16	8	2245
5c	60	36	1320	234	154	2862	1436	1186	6743	510	366	2671
6c	3	92	422	11	334	1267	158	1747	4635	30	686	1026
8c	96	73	1881	350	280	3624	1849	1636	7392	722	600	3474
9c	2	2	1275	6	6	2950	82	75	7529	16	15	2670

^a These parameters were determined by ELISA BrdU Assay.

Table 7
% Cytotoxicity of **2a-c**, **3a-c**, **5a-c**, **6a-c**, **8a-c**, **9a-c** at IC₅₀ concentrations against A549, Hep3B, MCF7, C6, HeLa, HT29, and FL.

Compound	A549	Hep3B	MCF7	C6	HeLa	HT29	FL
2a	15.52	19.57	12.79	11.96	26.92	15.40	14.76
2b	15.64	22.80	13.68	30.08	24.33	22.55	18.68
2c	23.43	21.28	16.47	27.68	26.22	20.58	22.29
3a	17.29	16.53	8.80	15.65	19.39	15.48	20.84
3b	14.63	19.82	17.10	22.49	23.25	22.61	16.53
3c	19.25	18.56	18.11	29.07	19.32	20.71	18.68
5a	23.50	19.63	15.39	21.41	22.67	21.41	15.39
5b	21.03	17.73	15.52	27.55	18.37	21.66	24.83
5c	25.46	18.05	24.76	25.71	24.45	22.04	20.52
6a	14.82	7.60	17.16	14.32	23.25	21.60	24.13
6b	25.97	14.12	18.56	26.16	22.74	18.87	19.32
6c	16.78	16.28	24.64	27.80	20.90	19.51	16.47
8a	18.49	12.41	17.35	22.11	27.61	20.90	16.35
8b	26.09	11.27	15.96	23.06	14.44	16.91	25.14
8c	23.50	19.38	16.66	29.32	20.14	22.55	18.18
9a	15.96	8.42	5.57	28.75	19.32	24.45	23.56
9b	14.19	21.91	13.87	25.78	14.33	25.97	26.22
9c	24.07	21.03	16.02	23.38	19.51	23.56	18.94

Table 8
% Cytotoxicity of positive controls at IC₅₀ concentrations.

	HeLa	HT29	A549	MCF7	C6	Hep3B	FL
Cisplatin	9.85	11.23	8.63	10.71	9.04	8.46	8.33
5FU	8.83	7.91	9.19	7.69	10.01	9.67	8.44

Table 9
% Cytotoxicity of **1–9** at IC₅₀ concentrations against C6, HeLa, and HT29.

Compound	C6	HeLa	HT29
1	20.52	19.95	20.71
2	25.39	26.60	25.08
3	19.63	12.54	24.76
4	19.89	10.64	20.39
5	10.39	22.99	22.10
6	22.30	21.47	20.91
7	18.56	9.56	21.16
8	15.83	21.16	24.07
9	23.05	22.36	17.29

in the human body is very important in this regard. Those that have MIC values of less than 125 µg/mL and less than our test molecules were evaluated as antibacterial. This evaluation was made according to the MIC values of antimicrobial drugs used as positive controls. Our test compounds showed highly antimicrobial activity against the Gram (+) bacteria described below. When the MIC values of newly synthesized

compounds displayed on Gram (+) bacteria were examined, their antibacterial effects were found to be more or similar to the SCF (sulbactam (30 µg) + cefoperazone (75 µg)) antibiotic used as a positive control against *S. gordonii* (NCTC 7870) for **2b-c**, **3a-c**, **5a**, **6b-c**, **8b-c**, and **9a-c** (< 7.81–125 µg/mL), against *S. mutans* (ATCC 35668) for **1**, **2**, **3**, **4**, **5**, **6**, **8**, **2a-c**, **3a-c**, **5a-c**, **6b-c**, **8a-c**, and **9a-c**, against *S. aureus* (MRSA ATCC 46300) for **2a-c**, **3a-c**, **5a-c**, **6b-c**, **8a-c**, and **9a-c** (< 7.81–125 µg/ml), against *S. aureus* (MSSA ATCC 29213) for **1**, **2**, **2a-c**, **3a-c**, **5a-c**, **6b-c**, **8a-c**, and **9a-c** (< 7.81–125 µg/ml), against *S. aureus* (ATCC 25923) for **2a-c**, **3a-c**, **5a-c**, **6b-c**, **8a-c**, and **9a-c**, against *E. faecalis* (ATCC 29212) for **2a-c**, **3b-c**, **5a-c**, **6b-c**, **8b-c**, and **9b-c** (< 7.81–125 µg/mL) and against *E. faecalis* (VRE ATCC 19433) for **6**, **9**, **2c**, **5b-c**, **6b-c**, and **8a** (31.25–125 µg/mL) (Tables 10–12).

Our test compounds showed sufficient antimicrobial activities against the Gram (–) bacteria mentioned below, but the effects of these antimicrobial activities were not as high as in Gram (+) bacteria. When the MIC values of the newly synthesized compounds displayed on the Gram (–) bacteria were examined, they had the same sensitivity as the SCF antibiotic used as a positive control against *A. actinomycetemcomitans* (ATCC 33384) for compounds **2**, **2a-c**, **3a-c**, **5a-c**, **6b-c**, **8c**, and **9b-c** (< 7.81–125 µg/mL), against *E. coli* (ESBL ATCC 35218) for compounds **2b**, **3b**, **5b**, **6b**, **8b**, and **9b** (15.62–62.5 µg/mL) and against *P. aeruginosa* (AGME ATCC 27853) for compounds **2b**, **3b**, **5b**, **6b**, and **9a** (31.25–125 µg/mL) (Tables 10–12). However, none of our new molecules have achieved a sufficiently strong antibacterial effect on the *E. coli* ATCC 25922 strain. The results showed that when the values of the in vitro antibacterial tests on compounds were evaluated, *N*-alkyl bromide derivatives (**a-c series**) exhibited stronger antibacterial properties than their parent molecules, 2,4,6-trisubstituted pyrimidines (**1–9**). It is also interesting that both groups of molecules exhibited antibacterial activities on relatively more Gram (+) bacteria. The most important point is that the strong antimicrobial activities against resistant strains such as VRE, MRSA, ESBL and AGME are much better and more desired than the control antibacterial drug SCF. In general, it can be said that alkyl derivatives have an especially strong effect and are very suitable for advanced pharmacological researches on some pathogen that cause disease in the human body on both groups of molecules.

2.2.4. Characteristics of DNA/BSA binding properties of molecules

Before advanced pharmacological testing of newly synthesized drug candidate molecules in pharmaceutical chemistry, physical interactions with biomacromolecules such as DNA or protein must be tried using various methods. Spectrophotometric techniques come first because of the easy and high accuracy of the methods used for this purpose. The interaction of candidate drug molecules with DNA or protein causes significant changes in the structure of these biomolecules and can be observed with spectrophotometric techniques. These significant

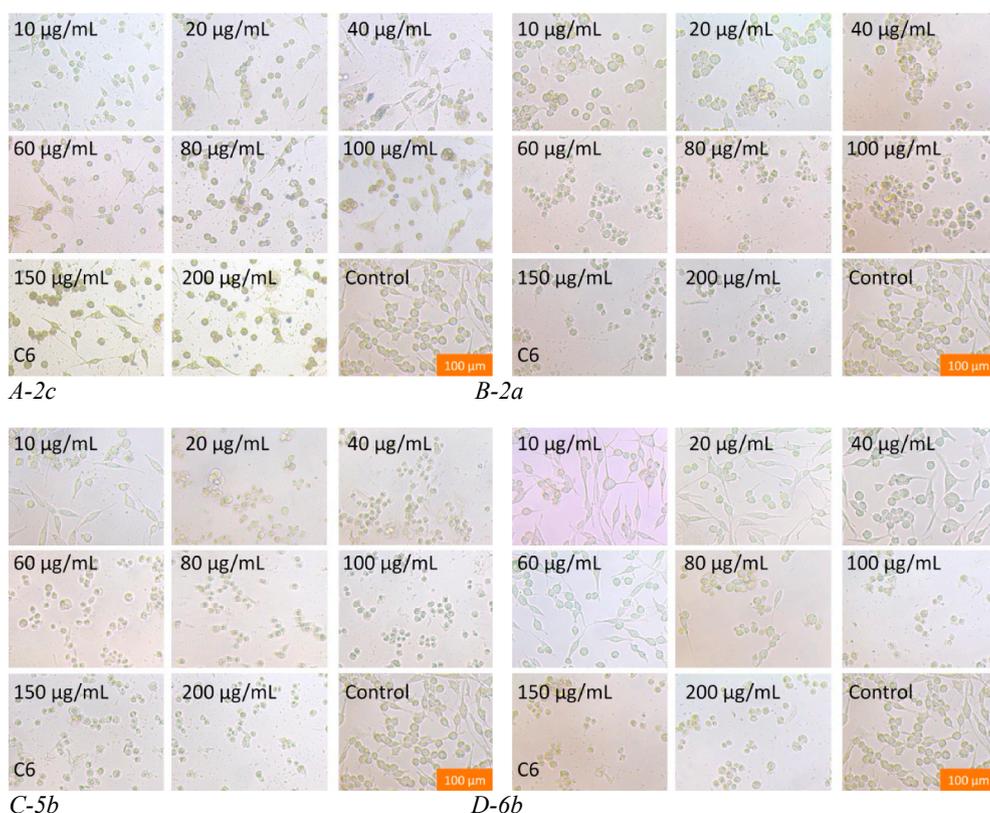


Fig. 2. Effect of **A-2c**, **B-2a**, **C-5b** and **D-6b** on the morphologies of C6 cell lines. Exponentially growing cells were incubated overnight with various concentrations of these molecules at 37 °C. Control cells were treated with only DMSO. All measurements were 100 µm.

spectral changes caused by candidate drug molecules on DNA and protein show up as a hyperchromic or hypochromic effect on the absorption spectra [37]. If the increase or the decrease in the absorption band occurs when DNA or protein is added at increasing concentrations to the constant concentrations of the molecule tested, it is called a hyperchromic effect and hypochromic effect, respectively. The hypochromic effect is usually an indication that the complex is bound to the DNA by the electrostatic action or often intercalated. In addition to this change in the spectra, the red or blue shift in the absorption bands of the complexes may be a sign of the stability of the complex-DNA structure. The DNA / BSA binding properties of newly synthesized chemicals synthesized were determined using a UV–vis spectrophotometer. The interactions of these compounds with DNA are as follows: In the UV–vis spectra of newly synthesized compounds, a single

maximum absorption peak was observed and on this peak, there was no clear bathochromic or hypsochromic effect with the exception of compounds 3, 5 and 6. When CT-DNA is added in increasing quantities to the reaction mixture, the reduction in the absorption intensity of compounds 1 and 4 resulted in hypochromic effect and the increase in the absorption intensity of compounds 2, 7, 8 and 9 caused hyperchromic appearance. Compounds 3, 5 and 6 also caused redshift about 10 nm when they evaluated for their possible physical relationships with DNA and showed hyperchromic properties, unlike the others. Likewise, when CT-DNA was added in increasing amount to the reaction tube, compounds 2a, 2c, 3b, 5c, 6a, 6c, 8a, 9b, and 9c caused a hyperchromic effect, whereas compounds 2b, 3a, 3c, 5a, 5b, 6b, 8b, 8c, and 9a from the same group showed a hypochromic action. The spectrum bands described below will be the guide lines to predict

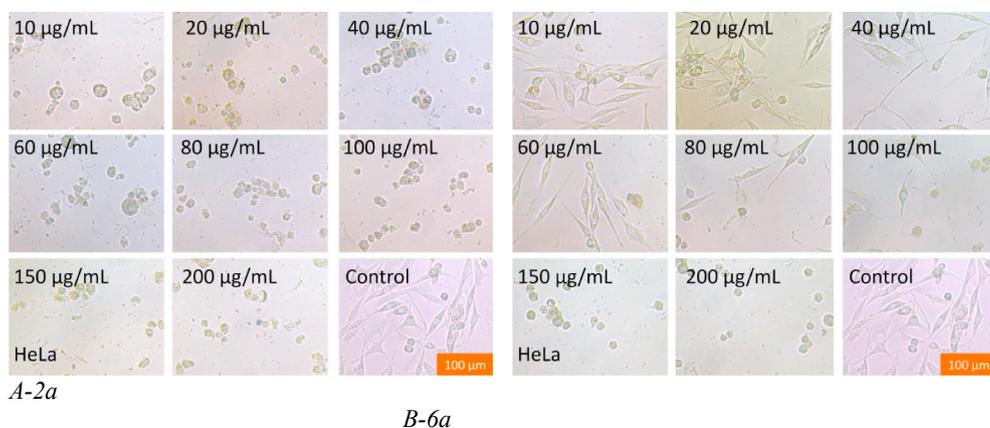


Fig. 3. Effect of **A-2a** and **B-6a** on the morphologies of HeLa cell lines. Exponentially growing cells were incubated overnight with various concentrations of these molecules at 37 °C. Control cells were treated with only DMSO. All measurements were 100 µm.

Table 10
Minimum-inhibitory concentrations (MIC, in µg/mL) of 1–9.

Microorganisms	1	2	3	4	5	6	7	8	9	KCN	SCF
<i>E. faecalis</i> VRE ATCC 19433	500	1000	1000	1000	500	125	250	500	125	NE	250
<i>E. faecalis</i> ATCC 29212	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	NE	62.5
<i>S. aureus</i> ATCC 25923	500	1000	1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	NE	250
<i>S. aureus</i> MSSA ATCC 29213	125	125	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	NE	NA
<i>S. aureus</i> MRSA ATCC 46300	> 1000	1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	NE	250
<i>E. coli</i> ATCC 25922	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	NE	15.62
<i>E. coli</i> ESBL ATCC 35218	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	NE	31.25
<i>P. aeruginosa</i> AGME ATCC 27853	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	NE	250
<i>S. mutans</i> ATCC 35668	125	62.5	125	125	62.5	62.5	500	125	500	NE	125
<i>S. gordonii</i> NCTC 7870	500	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	1000	NE	125
<i>A. actinomycetemcomitans</i> ATCC 33384	500	125	1000	> 1000	1000	> 1000	> 1000	> 1000	1000	NE	62.5

SCF, sulbactam (30 µg) + cefoperazone (75 µg), as a positive control.

KCN, potassium cyanide, as a negative control, NE, No effect NA, Not available.

Table 11
Minimum-inhibitory concentrations (MIC, in µg/mL) of 2a-c, 3a-c, 5a-c.

Microorganisms	2a	2b	2c	3a	3b	3c	5a	5b	5c	KCN	SCF
<i>E. faecalis</i> VRE ATCC 19433	250	1000	62.5	500	250	> 1000	1000	31.25	62.5	NE	250
<i>E. faecalis</i> ATCC 29212	125	< 7.81	62.5	250	< 7.81	62.5	62.5	< 7.81	< 7.81	NE	62.5
<i>S. aureus</i> ATCC 25923	62.5	< 7.81	62.5	125	< 7.81	125	125	< 7.81	< 7.81	NE	250
<i>S. aureus</i> MSSA ATCC 29213	62.5	< 7.81	< 7.81	62.5	< 7.81	< 7.81	< 7.81	< 7.81	< 7.81	NE	NA
<i>S. aureus</i> MRSA ATCC 46300	62.5	< 7.81	62.5	125	< 7.81	62.5	15.62	< 7.81	< 7.81	NE	250
<i>E. coli</i> ATCC 25922	250	> 1000	> 1000	500	> 1000	> 1000	250	> 1000	> 1000	NE	15.62
<i>E. coli</i> ESBL ATCC 35218	250	15.62	1000	1000	15.62	> 1000	250	31.25	1000	NE	31.25
<i>P. aeruginosa</i> AGME ATCC 27853	500	31.25	> 1000	1000	62.5	> 1000	> 1000	125	> 1000	NE	250
<i>S. mutans</i> ATCC 35668	< 7.81	< 7.81	< 7.81	31.25	< 7.81	< 7.81	15.62	< 7.81	< 7.81	NE	125
<i>S. gordonii</i> NCTC 7870	250	< 7.81	15.62	62.5	62.5	31.25	62.5	> 1000	> 1000	NE	125
<i>A. actinomycetemcomitans</i> ATCC 33384	125	< 7.81	62.5	125	< 7.81	125	31.25	< 7.81	31.25	NE	62.5

SCF, sulbactam (30 µg) + cefoperazone (75 µg), as a positive control.

KCN, potassium cyanide, as a negative control, NE, No effect NA, Not available.

possible interactions of newly synthesized drug candidate molecules with BSA. The absorption spectra of alkyl derivatives, upon increasing the concentration of BSA, showed gradually decreases in the peak intensities for compounds **2a**, **2b**, **3a**, **5b** and **8b** and increases in the peak intensities for compounds **2c**, **3b**, **3c**, **5c**, **6b**, **6c** and **8c**.

The binding constants (Kb) of these complexes with CT-DNA were revealed using spectrophotometric techniques which can be applied easily in research laboratories with the help of Benesi-Hildebrand equality shown below [38]: $[DNA]/(\epsilon a - \epsilon f) = [DNA]/(\epsilon b - \epsilon f) + 1/Kb$ ($\epsilon b - \epsilon f$). The [DNA] symbol in this equation is the DNA concentration in the base pairs, the symbols ϵa , ϵf and ϵb are the molar absorption coefficients of $A_{observed}/[complex]$, free complex and complex-DNA solutions, respectively. Kb is the binding constant related to compound able to bind to DNA and it can be calculated algebraically according to

the slope of a line between $[DNA]/(\epsilon a - \epsilon f)$ and $[DNA]$. When the binding constants given in Table 13 were examined, it was seen that the Kb values of the newly synthesized 2,4,6-trisubstituted pyrimidines were between 2.0×10^4 and $8.9 \times 10^4 M^{-1}$. The binding constants of the molecules in this group were ordered from small to large as follows: **2** > **7** > **9** > **3** > **1** > **5** > **8** > **6** > **4**.

When the binding constants of alkyl derivatives were examined in the same table, it was understood that Kb values were 2.0×10^4 to $2.4 \times 10^5 M^{-1}$ and their DNA binding constants order was as **3c** > **2a** > **3b** > **3a** > **2b** > **6a** > **5b** > **6b** > **5a** > **2c** > **9a** > **8c** > **5c** > **9b** > **8a** > **9c** > **6c** > **8b**. High Kb values indicate that the corresponding complexes strongly bind to DNA. According to the CT-DNA binding studies performed with cisplatin and 5FU anticancer drugs in the literature, the cisplatin binding constant was

Table 12
Minimum-inhibitory concentrations (MIC, in $\mu\text{g}/\text{mL}$) of **6a-c**, **8a-c**, **9a-c**.

Microorganisms	6a	6b	6c	8a	8b	8c	9a	9b	9c	KCN	SCF
<i>E. faecalis</i> VRE ATCC 19433	1000	31.25	62.5	125	> 1000	> 1000	500	1000	1000	NE	250
<i>E. faecalis</i> ATCC 29212	500	< 7.81	31.25	250	< 7.81	15.62	500	< 7.81	15.62	NE	62.5
<i>S. aureus</i> ATCC 25923	250	< 7.81	125	125	15.62	250	125	250	15.62	NE	250
<i>S. aureus</i> MSSA ATCC 29213	250	< 7.81	< 7.81	31.25	62.5	31.25	125	< 7.81	31.25	NE	NA
<i>S. aureus</i> MRSA ATCC 46300	250	< 7.81	< 7.81	62.5	< 7.81	< 7.81	125	< 7.81	< 7.81	NE	250
<i>E. coli</i> ATCC 25922	1000	> 1000	> 1000	500	> 1000	> 1000	250	> 1000	> 1000	NE	15.62
<i>E. coli</i> ESBL ATCC 35218	1000	15.62	> 1000	500	62.5	> 1000	500	15.62	> 1000	NE	31.25
<i>P. aeruginosa</i> AGME ATCC 27853	> 1000	125	> 1000	1000	250	> 1000	125	250	> 1000	NE	250
<i>S. mutans</i> ATCC 35668	31.25	< 7.81	31.25	62.5	< 7.81	< 7.81	125	< 7.81	31.25	NE	125
<i>S. gordonii</i> NCTC 7870	500	31.25	62.5	250	< 7.81	< 7.81	62.5	125	< 7.81	NE	125
<i>A. actinomycetemcomitans</i> ATCC 33384	250	< 7.81	31.25	250	500	125	250	15.62	125	NE	62.5

SCF, sulbactam (30 μg) + cefoperazone (75 μg), as a positive control.

KCN, potassium cyanide, as a negative control, NE, No effect NA, Not available.

Table 13
The binding constants (K_b) of these compounds.

No	K_b (M^{-1})	No	K_b (M^{-1})	No	K_b (M^{-1})
1	6.3×10^4	2a	1.9×10^5	6a	9.4×10^4
2	8.9×10^4	2b	9.5×10^4	6b	7.9×10^4
3	6.5×10^4	2c	4.7×10^4	6c	2.2×10^4
4	2.0×10^4	3a	9.9×10^4	8a	3.1×10^4
5	5.8×10^4	3b	1.4×10^5	8b	2.0×10^4
6	3.3×10^4	3c	2.4×10^5	8c	4.4×10^4
7	8.2×10^4	5a	5.9×10^4	9a	4.5×10^4
8	4.8×10^4	5b	8.0×10^4	9b	3.5×10^4
9	6.6×10^4	5c	4.0×10^4	9c	2.6×10^4

reported to be $5.73 \times 10^4 \text{ M}^{-1}$ and the 5FU binding constant to be $9.7 \times 10^4 \text{ M}^{-1}$ [39,40]. When we compared the DNA binding affinity of these two anticancer drugs used clinically with the interest of the DNA binding of the tested molecules, it was found that the newly synthesized molecules bound sufficiently tightly to the DNA. When the data in Table 13 was examined, it reveals that the newly synthesized molecules, especially compound **2a**, **3a**, **3b**, and **3c**, bound DNA much more strongly than cisplatin and 5FU anticancer drugs.

3. Conclusion

In the present paper, synthetic procedure of a new series of 2,4,6-trisubstituted pyrimidines (**1–9**) and their alkyl derivatives (**2a-c**, **3a-c**, **5a-c**, **6a-c**, **8a-c**, **9a-c**) were described. A total of 27 compounds have been synthesized and only compounds **4**, **7** and **9** are known in the literature [17,24] but no studies have been conducted on their anticancer and antimicrobial properties. In addition, anti-proliferative, cytotoxic, antibacterial activities and DNA/protein binding affinities for the all synthetic compounds (**1–9**, **2a-c**, **3a-c**, **5a-c**, **6a-c**, **8a-c**, **9a-c**) were investigated. In general, many of the obtained compounds showed anti-proliferative effects on tested cancer cell lines. Alkylated derivatives, especially all compounds of **c** series, have exceptional efficacy. The anticancer results showed that the elongation of the alkyl chain has a detrimental effect on cancer cell lines. On the other hand, all test compounds exhibited highly antimicrobial activities against Gram (+) and Gram (–) bacteria but. Gram (+) bacteria has the strongest activity. In particular, *N*-alkyl bromide derivatives (**a-c** series) exhibited

stronger antibacterial properties than their parent molecules 2,4,6-trisubstituted pyrimidines (**1–9**). DNA binding affinities for all the synthetic compounds (**1–9**, **2a-c**, **3a-c**, **5a-c**, **6a-c**, **8a-c**, **9a-c**) showed that all newly synthesized molecules bound DNA much stronger than cisplatin and 5FU anticancer drugs. The conclusion leads to these compounds being would be useful for the development of new anticancer drugs.

4. Experimental

4.1. Materials and equipment

All starting chemical reagents and solvents used in the synthesis, purification and biological activity investigations were high grade commercial products purchased from Aldrich, Fluka, Sigma, Merck, Amresco, Carlo-Erba, Lonza, Roche and used without further purification. Thin-layer chromatography (TLC) and column chromatography were performed on Merck precoated 60 Kieselgel F_{254} analytical aluminum acidic plates and silica gel 60 (0.040{0.063 mm}), respectively. All reactions were monitored using TLC. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR in CDCl_3 , $\text{CDCl}_3/\text{CD}_3\text{OD}$, CD_3OD with tetramethyl-silane (TMS) as an internal standard. The elemental analyses were performed by a Costech ECS 4010 instrument. The mass spectral analyses were performed on a Micromass Quattro LC-MS/MS spectrophotometer. Infrared spectra were obtained using a PerkinElmer 1600FT-IR (4000–400 cm^{-1}) spectrometer. Melting points were determined using a Stuart SMP10 apparatus.

4.2. Methods

The synthesis of known compounds **i-ix** were performed according to the previously reported procedure [32,41].

4.2.1. General procedure for synthesis of compounds **1–9**

Methoxy substituted azachalcone (10 mmol), guanidine hydrochloride (10 mmol) and NaOH (15 mmol) were mixed in a flask and 20 mL absolute ethanol was added to the flask. The reaction mixture was refluxed for 6–12 h [5,11–14,19,20,22,25,28] with the control of progress by TLC examination and allowed to cool to room temperature after completion. Water was added to the completed reactions and

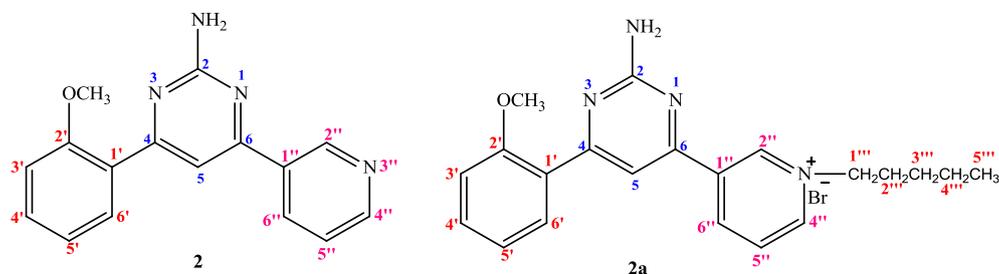


Fig. 4. Numbering of atoms on compounds **2** and **2a**.

pyrimidine was precipitated. The solid product was washed with cold acetone and then water. The filtered product was dried with a freeze dryer and the purity was checked with TLC. If necessary, it was purified by column chromatography and the structure was confirmed by spectroscopic methods (^1H , ^1H - ^1H COSY and ^{13}C -APT NMR, LC-MS/MS and FT-IR) and elemental analysis. Numbering of atoms in **2** and **2a** was given in Fig. 4 as example for spectroscopic analysis of pyrimidines and alkylated forms.

4.2.1.1. 4-(2-methoxyphenyl)-6-(pyridin-2-yl)pyrimidin-2-amine (1). Yield: 69%. M.p.: 170–172 °C. Rf: 0.40. (Hexane/diethyl ether 1:2).

FT-IR (cm^{-1}): 3297, 3169, 3004, 1626, 1604, 1536, 1453, 1247, 751, 745.

^1H NMR (400 MHz, CDCl_3 , ppm): δ = 8.2 (s, 1H, H-5); δ = 7.0 (d, J = 8.1 Hz, H-3'); δ = 7.4 (t, J = 8.0 Hz, 1H, H-4'); δ = 7.1 (t, J = 7.6 Hz, 1H, H-5'); δ = 7.8 (m, 1H, H-6'); δ = 8.7 (d, J = 4.8 Hz, 1H, H-3''); δ = 7.3 (t, J = 7.6/4.8 Hz, 1H, H-4''); δ = 7.8 (m, 1H, H-5''); δ = 8.3 (d, J = 8.0 Hz, 1H, H-6''); δ = 5.2 (bs, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, CDCl_3 , ppm): 166.0, 163.7, 163.3, 157.7, 155.1, 149.4, 136.8, 131.1, 130.6, 127.4, 124.7, 121.7, 120.8, 111.4, 109.3, 55.7.

Poz. LC-MS/MS m/z (%): 301(100) [$\text{M} + \text{Na}$] $^+$, 279 (30) [$\text{M} + 1$] $^+$.

Anal. cal. for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}$ (278.31 g/mol): C 69.05, H 5.07, N 20.13, found: C 69.02, H 5.09, N 20.12.

4.2.1.2. 4-(2-methoxyphenyl)-6-(pyridin-3-yl)pyrimidin-2-amine (2). Yield: 70%. M.p.: 96–98 °C. Rf: 0.47 (Hexane/diethyl ether 1:2).

FT-IR (cm^{-1}): 3277, 3080, 1660, 1543, 1244, 1023, 754, 633.

^1H NMR (400 MHz, CDCl_3 , ppm): δ = 7.7 (s, 1H, H-5); δ = 7.1 (d, J = 8.0 Hz, H-3'); δ = 7.5 (m, 1H, H-4'); δ = 7.1 (t, J = 8.0 Hz, 1H, H-5'); δ = 7.9 (d, J = 8.0 Hz, 1H, H-6'); δ = 9.3 (s, 1H, H-2''); δ = 8.7 (dd, J = 6.0 Hz, 1H, H-4''); δ = 7.4 (m, 1H, H-5''); δ = 8.4 (d, J = 8.0 Hz, 1H, H-6''); δ = 5.3 (bs, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, CDCl_3 , ppm): 166.0, 163.7, 163.3, 157.7, 155.1, 149.4, 136.8, 131.1, 130.6, 127.4, 124.7, 121.7, 120.8, 111.4, 109.3, 55.7.

Poz. LC-MS/MS m/z (%): 279 (100) [$\text{M} + 1$] $^+$.

Anal. cal. for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}$ (278.31 g/mol): C 69.05, H 5.07, N 20.13, found: C 69.03, H 5.10, N 20.12.

4.2.1.3. 4-(2-Methoxyphenyl)-6-(pyridin-4-yl)pyrimidin-2-amine (3). Yield: 68%. M.p.: 185–187 °C. Rf: 0.44 (Hexane/diethyl ether 1:2).

FT-IR (cm^{-1}): 3310, 3141, 2987, 1648, 1578, 1525, 1245, 823, 744, 622.

^1H NMR (400 MHz, CDCl_3 , ppm): δ = 7.7 (s, 1H, H-5); δ = 7.1 (d, J = 8.0 Hz, H-3'); δ = 7.5 (t, J = 8.0 Hz, 1H, H-4'); δ = 7.1 (t, J = 8.0 Hz, 1H, H-5'); δ = 7.9 (d, J = 8.0 Hz, 1H, H-6'); δ = 7.9 (d, J = 6.0 Hz, 2H, H-2''/6''); δ = 8.8 (d, J = 6.0 Hz, 2H, H-3''/5''); δ = 5.4 (bs, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, CDCl_3 , ppm): 165.8, 163.5, 162.4, 157.7, 150.4, 145.3, 131.5, 130.7, 126.7, 121.2, 121.1, 111.6, 109.2, 55.7.

Poz. LC-MS/MS m/z (%): 106 (100) [Methoxyphenyl-1] $^+$, 279 (80) [$\text{M} + 1$] $^+$.

Anal. cal. for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}$ (278.31 g/mol): C 69.05, H 5.07, N 20.13, found: C 69.02, H 5.08, N 20.11.

4.2.1.4. 4-(3-Methoxyphenyl)-6-(pyridin-2-yl)pyrimidin-2-amine (4). Yield: 74%. M.p.: 157–159 °C (lit. [28] 131–132 °C). Rf: 0.53 (Hexane/diethyl ether 1:2).

FT-IR (cm^{-1}): 3371, 3313, 3184, 2986, 1633, 1538, 1359, 1044, 774.

^1H NMR (400 MHz, CDCl_3 , ppm): δ = 8.2 (s, 1H, H-5); δ = 7.4 (m, 1H, H-2'); δ = 7.1 (d, J = 8.0 Hz, 1H, H-4'); δ = 7.4 (m, 1H, H-5'); δ = 7.7 (d, J = 8.0 Hz, 1H, H-6'); δ = 8.7 (d, J = 4.8 Hz, 1H, H-3''); δ = 7.4 (m, 1H, H-4''); δ = 7.8 (t, J = 8.0 Hz, 1H, H-5''); δ = 8.4 (d, J = 8.0 Hz, 1H, H-6''); δ = 5.2 (bs, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, CDCl_3 , ppm): 166.4, 164.7, 163.4, 159.9, 154.7, 149.4, 139.0, 136.9, 129.7, 124.9, 121.6, 119.8, 116.7, 112.1, 104.6, 55.4.

Poz. LC-MS/MS m/z (%): 279 (100) [$\text{M} + 1$] $^+$.

Anal. cal. for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}$ (278.31 g/mol): C 69.05, H 5.07, N 20.13, found: C 69.04, H 5.10, N 20.10.

4.2.1.5. 4-(3-Methoxyphenyl)-6-(pyridin-3-yl)pyrimidin-2-amine (5). Yield: 73%. M.p.: 164–166 °C. Rf: 0.41 (Hexane/diethyl ether 1:3).

FT-IR (cm^{-1}): 3385, 3263, 1636, 1556, 1527.

^1H NMR (400 MHz, CDCl_3 , ppm): δ = 7.5 (s, 1H, H-5); δ = 7.7 (s, 1H, H-2'); δ = 7.1 (d, J = 8.0 Hz, 1H, H-4'); δ = 7.4 (t, J = 8.0 Hz, 1H, H-5'); δ = 7.6 (d, J = 8.0 Hz, 1H, H-6'); δ = 9.3 (s, 1H, H-2''); δ = 8.8 (d, J = 4.8 Hz, 1H, H-4''); δ = 7.4 (t, J = 4.4 Hz, 1H, H-5''); δ = 8.4 (d, J = 8.0 Hz, 1H, H-6''); δ = 5.2 (s, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, CDCl_3 , ppm): 166.6, 163.6, 163.5, 160.0, 150.9, 148.2, 138.6, 134.8, 133.4, 129.8, 123.7, 116.7, 115.5, 112.3, 104.2, 55.0.

Poz. LC-MS/MS m/z (%): 106 (100) [Methoxyphenyl-1] $^+$, 279 (18) [$\text{M} + 1$] $^+$.

Anal. cal. for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}$ (278.31 g/mol): C 69.05, H 5.07, N 20.13, found: C 69.04, H 5.08, N 20.09.

4.2.1.6. 4-(3-Methoxyphenyl)-6-(pyridin-4-yl)pyrimidin-2-amine (6). Yield: 59%. M.p.: 154–156 °C. Rf: 0.52 (Hexane/diethyl ether 1:2).

FT-IR (cm^{-1}): 3502, 3324, 3077, 1632, 1560, 1531, 1260, 778.

^1H NMR (400 MHz, CDCl_3 , ppm): δ = 7.5 (s, 1H, H-5); δ = 7.6 (bs, H-2'); δ = 7.1 (d, J = 8.0 Hz, 1H, H-4'); δ = 7.4 (t, J = 8.0 Hz, 1H, H-5'); δ = 7.6 (d, J = 8.0 Hz, 1H, H-6'); δ = 7.9 (d, J = 8.0 Hz, 2H, H-2''/6''); δ = 8.8 (d, J = 8.0 Hz, 2H, H-3''/5''); δ = 5.3 (s, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, CDCl_3 , ppm): 167.3, 164.0, 164.0, 160.5, 150.7, 145.6, 139.0, 130.3, 121.6, 119.0, 117.1, 112.8, 104.9, 55.9.

Poz. LC-MS/MS m/z (%): 106 (100) [Methoxyphenyl-1] $^+$, 279 (23) [$\text{M} + 1$] $^+$.

Anal. cal. for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}$ (278.31 g/mol): C 69.05, H 5.07, N 20.13, found: C 69.07, H 5.08, N 20.11.

4.2.1.7. 4-(4-Methoxyphenyl)-6-(pyridin-2-yl)pyrimidin-2-amine (**7**). Yield: 61%. M.p.: 208–210 °C (lit. [28] 199–200 °C). Rf: 0.47 (Hexane/diethyl ether 1.5:2.5).

FT-IR (cm⁻¹): 3326, 3185, 1643, 1606, 1561, 1531, 1360, 1256, 1231, 787.

¹H NMR (400 MHz, CDCl₃, ppm): δ = 8.1 (s, 1H, H-5); δ = 8.2 (d, J = 8.0 Hz, 2H, H-2'/6'); δ = 7.0 (d, J = 8.0 Hz, 2H, H-3'/5'); δ = 8.7 (d, J = 4.8 Hz, 1H, H-3''); δ = 7.4 (t, J = 8.0 Hz, 1H, H-4''); δ = 7.8 (t, J = 8.0 Hz, 1H, H-5''); δ = 8.3 (d, J = 8.0 Hz, 1H, H-6''); δ = 5.3 (s, 2H, NH₂); 3.9 (s, 3H, -OCH₃).

¹³C NMR (100 MHz, CDCl₃, ppm): 166.3, 164.4, 163.4, 161.9, 154.9, 149.3, 137.1, 129.8, 128.9, 125.0, 121.5, 114.3, 103.7, 55.6.

Poz. LC-MS/MS *m/z* (%): 109 (1 0 0) [Methoxybenzene + 1]⁺, 279 (70) [M + 1]⁺.

Anal. cal. for C₁₆H₁₄N₄O (278.31 g/mol): C 69.05, H 5.07, N 20.13, found: C 69.04, H 5.09, N 20.12.

4.2.1.8. 4-(4-Methoxyphenyl)-6-(pyridin-3-yl)pyrimidin-2-amine (**8**). Yield: 77%. M.p.: 161–163 °C. Rf: 0.50 (Hexane/diethyl ether 1:3).

FT-IR (cm⁻¹): 3457, 3328, 3210, 3188, 1644, 1540, 1515, 1364, 1240, 1023, 803.

¹H NMR (400 MHz, CDCl₃, ppm): δ = 7.5 (s, 1H, H-5); δ = 8.1 (d, J = 8.0 Hz, 2H, H-2'/6'); δ = 7.1 (d, J = 8.0 Hz, 2H, H-3'/5'); δ = 9.3 (d, J = 2.0 Hz, 1H, H-2''); δ = 8.8 (dd, J = 4.8 Hz, 1H, H-4''); δ = 7.5 (t, J = 8.0 Hz, 1H, H-5''); δ = 8.4 (d, J = 8.0 Hz, 1H, H-6''); δ = 5.2 (s, 2H, NH₂); 3.9 (s, 3H, -OCH₃).

¹³C NMR (100 MHz, CDCl₃, ppm): 166.1, 163.6, 163.3, 161.9, 151.1, 148.4, 134.5, 133.5, 130.0, 128.9, 123.7, 114.3, 103.5, 55.4.

Poz. LC-MS/MS *m/z* (%): 106 (100) [Methoxyphenyl-1]⁺, 279 (12) [M + 1]⁺.

Anal. cal. for C₁₆H₁₄N₄O (278.31 g/mol): C 69.05, H 5.07, N 20.13, found: C 69.04, H 5.10, N 20.11.

4.2.1.9. 4-(4-Methoxyphenyl)-6-(pyridin-4-yl)pyrimidin-2-amine (**9**). Yield: 67%. M.p.: 206–208 °C (lit. [11] 128–130 °C). Rf: 0.45 (Hexane/diethyl ether 1.5:2.5).

FT-IR (cm⁻¹): 3326, 3188, 1643, 1606, 1581, 1561, 1531.

¹H NMR (400 MHz, CDCl₃, ppm): δ = 7.5 (s, 1H, H-5); δ = 8.1 (d, J = 8.8 Hz, 2H, H-2'/6'); δ = 7.0 (d, J = 8.8 Hz, 2H, H-3'/5'); δ = 7.9 (d, J = 6.4 Hz, 2H, H-2''/6''); δ = 8.8 (d, J = 6.4 Hz, 2H, H-3''/5''); δ = 5.2 (s, 2H, NH₂); 3.9 (s, 3H, -OCH₃).

¹³C NMR (100 MHz, CDCl₃, ppm): 166.4, 163.2, 162.1, 150.2, 145.5, 129.4, 128.7, 121.2, 114.3, 103.7, 55.4.

Poz. LC-MS/MS *m/z* (%): 279 (100) [M + 1]⁺.

Anal. cal. for C₁₆H₁₄N₄O (278.31 g/mol): C 69.05, H 5.07, N 20.13, found: C 69.03, H 5.09, N 20.12.

4.2.2. General procedure for synthesis of compounds **2a-c**, **3a-c**, **5a-c**, **6a-c**, **8a-c**, **9a-c**

Pyrimidines (**2**, **3**, **5**, **6**, **8**, **9**) as starting materials (~5.0 mmol for each) were dissolved in 15 mL of acetonitrile separately. 1-Bromopentane (**a series**), 1-bromodecane (**b series**), and 1-bromopentadecane (**c series**), 5.0 mmol each) were added to reaction flasks one by one for every pyrimidines. All solutions were refluxed separately for 12–22 h [31–36]. On completion of the reaction, followed by TLC examination, the mixtures cooled to room temperature. The resulting lemon yellow coloured precipitates were filtered and washed first with hexane, then with ethyl acetate. Also some compounds were obtained in oily form. The purities of dried products were checked with TLC and structures were identified by spectroscopic methods.

4.2.2.1. 3-[2-Amino-6-(2-methoxyphenyl)pyrimidin-4-yl]-1-pentylpyridinium bromide (**2a**). Yield: 69%. Oil. Rf: 0.35 (Ethyl acetate/ methanol 1:1).

FT-IR (cm⁻¹): 3443, 3322, 3184, 3075, 2931, 2863, 1638, 1599, 1572, 1540, 1247, 756.

¹H NMR (400 MHz, CDCl₃, ppm): δ = 7.9 (s, 1H, H-5); δ = 7.1 (d,

J = 8.0 Hz, H-3''); δ = 7.5 (t, J = 8.0 Hz, 1H, H-4'); δ = 7.1 (t, J = 8.0 Hz, 1H, H-5''); δ = 7.9 (d, 8.0 Hz, 1H, H-6''); δ = 9.6 (s, 1H, H-2''); δ = 9.8 (d, J = 6.0 Hz, 1H, H-4''); δ = 8.3 (t, J = 6.0 Hz, 1H, H-5''); δ = 9.0 (d, J = 8.0 Hz, 1H, H-6''); δ = 5.1 (t, J = 8.0 Hz, 2H, H-1''); δ = 2.1 (m, 2H, H-2''); δ = 1.5–1.4 (m, 4H, H-3'''–4'''); δ = 0.9 (t, J = 8.0 Hz, 3H, H-5''); δ = 5.4 (s, 2H, NH₂); 4.0 (s, 3H, -OCH₃).

¹³C NMR (100 MHz, CDCl₃, ppm): 166.9, 163.3, 158.0, 156.8, 146.1, 142.7, 142.3, 138.3, 131.3, 130.8, 128.7, 125.7, 121.1, 111.7, 109.0, 62.8, 56.1, 31.7, 28.0, 22.1, 13.8.

Poz. LC-MS/MS *m/z* (%): 349 (1 0 0) [M - ⁷⁹Br/⁸¹Br]⁺, 350 (25) [M - ⁷⁹Br/⁸¹Br + 1]⁺.

Anal. cal. for C₂₁H₂₅BrN₄O (429.36 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.79, H 5.89, N 13.03.

4.2.2.2. 3-[2-Amino-6-(2-methoxyphenyl)pyrimidin-4-yl]-1-decylpyridinium bromide (**2b**). Yield: 80%. M.p.: 146–148 °C. Rf: 0.60 (Ethyl acetate/ methanol 2:1).

FT-IR (cm⁻¹): 3449, 3322, 3183, 3073, 2923, 2853, 1638, 1599, 1569, 1540, 1249, 750.

¹H NMR (400 MHz, CDCl₃, ppm): δ = 7.9 (s, 1H, H-5); δ = 7.0 (d, J = 8.0 Hz, H-3''); δ = 7.4 (t, J = 8.0 Hz, 1H, H-4'); δ = 7.1 (t, J = 8.0 Hz, 1H, H-5''); δ = 7.9 (d, J = 8.0 Hz, 1H, H-6''); δ = 9.7 (s, 1H, H-2''); δ = 9.6 (d, J = 6.0 Hz, 1H, H-4''); δ = 8.3 (t, J = 8.0 Hz, 1H, H-5''); δ = 9.0 (d, J = 8.0 Hz, 1H, H-6''); δ = 5.1 (t, J = 8.0 Hz, 2H, H-1''); δ = 2.1 (m, 2H, H-2''); δ = 1.3–1.2 (m, 14H, H-3'''–9'''); δ = 0.9 (t, J = 6.5 Hz, 3H, H-10''); δ = 5.6 (s, 2H, NH₂); 4.0 (s, 3H, -OCH₃).

¹³C NMR (100 MHz, CDCl₃, ppm): 163.3, 166.8, 158.0, 156.8, 145.8, 142.8, 142.4, 138.1, 132.2, 130.8, 128.7, 125.8, 121.0, 111.7, 109.0, 62.7, 56.1, 32.0, 31.8, 29.4, 29.3, 29.2, 29.1, 26.1, 22.6, 14.1.

Poz. LC-MS/MS *m/z* (%): 419 (1 0 0) [M - ⁷⁹Br/⁸¹Br]⁺, 420 (85) [M - ⁷⁹Br/⁸¹Br + 1]⁺.

Anal. cal. for C₂₆H₃₅BrN₄O (499.49 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.80, H 5.88, N 13.01.

4.2.2.3. 3-[2-Amino-6-(2-methoxyphenyl)pyrimidin-4-yl]-1-pentadecylpyridinium bromide (**2c**). Yield: 55%. M.p.: 153–155 °C. Rf: 0.52 (Ethyl acetate/ methanol 2:1).

FT-IR (cm⁻¹): 3451, 3323, 3183, 3073, 3003, 2920, 2851, 1639, 1599, 1571, 1540, 1250, 749.

¹H NMR (400 MHz, CDCl₃, ppm): δ = 7.9 (s, 1H, H-5); δ = 7.1 (d, J = 8.0 Hz, H-3''); δ = 7.5 (t, J = 8.0 Hz, 1H, H-4'); δ = 7.1 (t, J = 8.0 Hz, 1H, H-5''); δ = 7.9 (d, J = 8.0 Hz, 1H, H-6''); δ = 9.6 (s, 1H, H-2''); δ = 9.9 (d, J = 6.0 Hz, 1H, H-4''); δ = 8.3 (t, J = 8.0 Hz, 1H, H-5''); δ = 9.0 (d, J = 8.0 Hz, 1H, H-6''); δ = 5.2 (t, J = 8.0 Hz, 2H, H-1''); δ = 2.1 (m, 2H, H-2''); δ = 1.5–1.3 (m, 24H, H-3'''–14'''); δ = 0.9 (t, J = 8.0 Hz, 3H, H-15''); δ = 5.4 (s, 2H, NH₂); 4.0 (s, 3H, -OCH₃).

¹³C NMR (100 MHz, CDCl₃, ppm): 167.1, 163.4, 158.0, 156.7, 146.1, 142.6, 142.2, 138.3, 132.2, 130.8, 128.6, 125.8, 121.1, 111.7, 109.0, 62.8, 56.1 32.0, 31.9, 29.7, 29.6, 29.5, 29.4, 29.1, 26.1, 22.7, 14.1.

Poz. LC-MS/MS *m/z* (%): 489 (1 0 0) [M - ⁷⁹Br/⁸¹Br]⁺, 490 (95) [M - ⁷⁹Br/⁸¹Br + 1]⁺.

Anal. cal. for C₃₁H₄₅BrN₄O (569.63 g/mol): C 65.37, H 7.96, N 9.84, found: C 65.32, H 7.99, N 9.81.

4.2.2.4. 4-[2-Amino-6-(2-methoxyphenyl)pyrimidin-4-yl]-1-pentylpyridinium bromide (**3a**). Yield: 55%. M.p.: 82–84 °C. Rf: 0.39 (Ethyl acetate/ methanol 2:1).

FT-IR (cm⁻¹): 3324, 3205, 2956, 2932, 2863, 1637, 1581, 1541, 1450, 1360, 1247, 1014, 769.

¹H NMR (400 MHz, CDCl₃/CD₃OD, ppm): δ = 7.9 (s, 1H, H-5); δ = 7.0 (d, J = 8.0 Hz, H-3''); δ = 7.5 (t, J = 8.0 Hz, 1H, H-4'); δ = 7.1 (t, J = 8.0 Hz, 1H, H-5''); δ = 7.9 (bs, 1H, H-6''); δ = 8.6 (d, J = 5.6 Hz, 2H, H-2''/6''); δ = 9.3 (d, J = 5.6 Hz, 2H, H-3''/5''); δ = 4.9 (t, J = 8.0 Hz, 2H, H-1''); δ = 2.0 (m, 2H, H-2''); δ = 1.4 (bs, 4H, H-3'''–4'''); δ = 0.9 (t, J = 8.0 Hz, 3H, H-5''); δ = 5.6 (s, 2H, NH₂); 3.9 (s, 3H,

–OCH₃).

¹³C NMR (100 MHz, CDCl₃/CD₃OD, ppm): 167.1, 163.5, 157.8, 157.1, 153.3, 145.0, 132.2, 130.5, 125.6, 125.4, 121.0, 111.6, 109.7, 61.8, 55.7, 31.3, 28.0, 22.0, 13.6.

Poz. LC-MS/MS *m/z* (%): 349 (1 0 0) [M – ⁷⁹Br/⁸¹Br]⁺.

Anal. cal. for C₂₁H₂₅BrN₄O (429.36 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.80, H 5.88, N 13.09.

4.2.2.5. 4-[2-Amino-6-(2-methoxyphenyl)pyrimidin-4-yl]-1-decylpyridinium bromide (3b). Yield: 75%. M.p.: 88–90 °C. Rf: 0.38 (Ethyl acetate/methanol 2:1).

FT-IR (cm⁻¹): 3322, 3193, 2923, 2853, 1637, 1540, 1447, 1248, 1017, 754.

¹H NMR (400 MHz, CDCl₃/CD₃OD, ppm): δ = 7.9 (s, 1H, H-5); δ = 7.1 (d, *J* = 8.0 Hz, H-3'); δ = 7.5 (t, *J* = 8.0 Hz, 1H, H-4'); δ = 7.1 (t, *J* = 8.0 Hz, 1H, H-5'); δ = 7.9 (d, *J* = 8.0 Hz, 1H, H-6'); δ = 8.7 (d, *J* = 6.8 Hz, 2H, H-2''/6''); δ = 9.3 (d, *J* = 6.8 Hz, 2H, H-3''/5''); δ = 4.9 (t, *J* = 8.0 Hz, 2H, H-1'''); δ = 2.0 (m, 2H, H-2'''); δ = 1.4–1.3 (m, 14H, H-3'''–9'''); δ = 0.9 (t, *J* = 8.0 Hz, 3H, H-10'''); δ = 5.7 (s, 2H, NH₂); 4.0 (s, 3H, –OCH₃).

¹³C NMR (100 MHz, CDCl₃/CD₃OD, ppm): 166.9, 163.4, 158.0, 157.1, 153.1, 145.1, 132.4, 130.7, 125.4, 125.3, 121.1, 111.6, 109.0, 61.9, 55.9, 31.8, 29.4, 29.3, 29.2, 29.0, 26.1, 22.6, 14.0.

Poz. LC-MS/MS *m/z* (%): 420 (1 0 0) [M – ⁷⁹Br/⁸¹Br + 1]⁺.

Anal. cal. for C₂₆H₃₅BrN₄O (499.49 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.87, H 5.84, N 13.04.

4.2.2.6. 4-[2-Amino-6-(2-methoxyphenyl)pyrimidin-4-yl]-1-pentadecylpyridinium bromide (3c). Yield: 50%. M.p.: 101–103 °C. Rf: 0.42 (Ethyl acetate/methanol 1:1).

FT-IR (cm⁻¹): 3434, 3316, 3198, 3039, 2921, 2849, 1636, 1541, 1247, 750.

¹H NMR (400 MHz, CDCl₃, ppm): δ = 7.9 (s, 1H, H-5); δ = 7.0 (d, *J* = 8.0 Hz, H-3'); δ = 7.5 (t, *J* = 8.0 Hz, 1H, H-4'); δ = 7.1 (t, *J* = 8.0 Hz, 1H, H-5'); δ = 7.9 (d, *J* = 8.0 Hz, 1H, H-6'); δ = 8.6 (d, *J* = 8.0 Hz, 2H, H-2''/6''); δ = 9.6 (d, *J* = 8.0 Hz, 2H, H-3''/5''); δ = 5.0 (t, *J* = 8.0 Hz, 2H, H-1'''); δ = 2.1 (m, 2H, H-2'''); δ = 1.3–1.2 (m, 24H, H-3'''–14'''); δ = 0.9 (t, *J* = 8.0 Hz, 3H, H-15'''); δ = 5.5 (s, 2H, NH₂); 4.0 (s, 3H, –OCH₃).

¹³C NMR (100 MHz, CDCl₃, ppm): 166.9, 163.5, 158.1, 157.0, 153.2, 145.4, 132.4, 130.8, 125.4, 125.2, 121.1, 111.6, 109.9, 61.8, 55.9, 31.9, 29.68, 29.67, 29.66, 29.65, 29.64, 29.63, 29.59, 29.50, 29.35, 29.08, 26.12, 22.68, 14.1.

Poz. LC-MS/MS *m/z* (%): 489 (1 0 0) [M – ⁷⁹Br/⁸¹Br]⁺, 490 (98) [M – ⁷⁹Br/⁸¹Br + 1]⁺.

Anal. cal. for C₃₁H₄₅BrN₄O (569.63 g/mol): C 65.37, H 7.96, N 9.84, found: C 65.33, H 8.1, N 9.83.

4.2.2.7. 3-[2-Amino-6-(3-methoxyphenyl)pyrimidin-4-yl]-1-pentylpyridinium bromide (5a). Yield: 58%. Oil. Rf: 0.47 (Ethyl acetate/methanol 1:1).

FT-IR (cm⁻¹): 3329, 3209, 2931, 1634, 1548, 1368, 1267, 1221, 791, 681.

¹H NMR (400 MHz, CDCl₃/CD₃OD, ppm): δ = 7.9 (s, 1H, H-5); δ = 7.7 (s, 1H, H-2'); δ = 7.1 (d, *J* = 8.0 Hz, 1H, H-4'); δ = 7.5 (t, *J* = 8.0 Hz, 1H, H-5'); δ = 7.8 (d, *J* = 8.0 Hz, 1H, H-6'); δ = 9.9 (s, 1H, H-2''); δ = 9.2 (d, *J* = 8.0 Hz, 1H, H-4'') δ = 8.2 (t, *J* = 8.0 Hz, 1H, H-5''); δ = 9.1 (d, *J* = 8.0 Hz, 1H, H-6''); δ = 4.9 (t, *J* = 8.0 Hz, 2H, H-1'''); δ = 2.1 (m, 2H, H-2'''); δ = 1.5–1.4 (m, 4H, H-3'''–4'''); δ = 1.0 (t, *J* = 6.4 Hz, 3H, H-5'''); δ = 5.8 (s, 2H, NH₂); 4.0 (s, 3H, –OCH₃).

¹³C NMR (100 MHz, CDCl₃/CD₃OD, ppm): 167.6, 163.7, 160.2, 158.1, 145.0, 143.5, 143.0, 138.2, 137.9, 130.1, 128.6, 120.3, 117.7, 112.5, 104.7, 62.7, 55.9, 31.8, 28.3, 22.3, 13.9.

Poz. LC-MS/MS *m/z* (%): MW = 428 (⁷⁹Br)/430 (⁸¹Br) g/mol; 349 (1 0 0) [M – ⁷⁹Br/⁸¹Br]⁺.

Anal. cal. for C₂₁H₂₅BrN₄O (429.36 g/mol): C 58.75, H 5.87, N

13.05, found: C 58.77, H 5.91, N 13.01.

4.2.2.8. 3-[2-Amino-6-(3-methoxyphenyl)pyrimidin-4-yl]-1-decylpyridinium bromide (5b). Yield: 78%. M.p.: 79–81 °C. Rf: 0.48 (Ethyl acetate/methanol 1:1).

FT-IR (cm⁻¹): 3495, 3306, 3176, 2924, 2854, 1625, 1570, 1543, 1463, 1367, 1233, 1040, 788.

¹H NMR (400 MHz, CDCl₃/CD₃OD, ppm): δ = 7.9 (s, 1H, H-5); δ = 7.7 (s, 1H, H-2'); δ = 7.0 (d, *J* = 8.0 Hz, 1H, H-4'); δ = 7.4 (t, *J* = 8.0 Hz, 1H, H-5'); δ = 7.8 (d, *J* = 8.0 Hz, 1H, H-6'); δ = 9.9 (s, 1H, H-2''); δ = 9.2 (d, *J* = 8.0 Hz, 1H, H-4'') δ = 8.1 (t, *J* = 8.0 Hz, 1H, H-5''); δ = 9.0 (d, *J* = 8.0 Hz, 1H, H-6''); δ = 4.9 (t, *J* = 8.0 Hz, 2H, H-1'''); δ = 2.0 (m, 2H, H-2'''); δ = 1.4–1.2 (m, 14H, H-3'''–9'''); δ = 0.9 (t, *J* = 8.0 Hz, 3H, H-10'''); δ = 5.8 (s, 2H, NH₂); 3.9 (s, 3H, –OCH₃).

¹³C NMR (100 MHz, CDCl₃/CD₃OD, ppm): 167.3, 163.5, 160.0, 157.9, 144.7, 143.3, 142.8, 137.9, 137.7, 130.0, 128.4, 120.1, 117.5, 112.3, 104.4, 62.5, 55.8, 31.9, 31.8, 29.5, 29.4, 29.2, 29.1, 26.1, 22.6, 14.0.

Poz. LC-MS/MS *m/z* (%): 419 (1 0 0) [M – ⁷⁹Br/⁸¹Br]⁺, 420 (89) [M – ⁷⁹Br/⁸¹Br + 1]⁺.

Anal. cal. for C₂₆H₃₅BrN₄O (499.49 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.77, H 5.90, N 13.07.

4.2.2.9. 3-[2-Amino-6-(3-methoxyphenyl)pyrimidin-4-yl]-1-pentadecylpyridinium bromide (5c). Yield: 50%. M.p.: 74–75 °C. Rf: 0.42 (Ethyl acetate/methanol 1:1).

FT-IR (cm⁻¹): 3334, 3206, 2921, 2851, 1633, 1569, 1570, 1548, 1508, 1465, 1223, 790, 681.

¹H NMR (400 MHz, CDCl₃/CD₃OD, ppm): δ = 7.9 (s, 1H, H-5); δ = 7.7 (s, 1H, H-2'); δ = 7.0 (d, *J* = 8.0 Hz, 1H, H-4'); δ = 7.4 (t, *J* = 8.0 Hz, 1H, H-5'); δ = 7.7 (d, *J* = 8.0 Hz, 1H, H-6'); δ = 9.9 (s, 1H, H-2''); δ = 9.2 (d, *J* = 8.0 Hz, 1H, H-4'') δ = 8.1 (t, *J* = 8.0 Hz, 1H, H-5''); δ = 9.0 (d, *J* = 6.0 Hz, 1H, H-6''); δ = 4.9 (t, *J* = 8.0 Hz, 2H, H-1'''); δ = 2.0 (m, 2H, H-2'''); δ = 1.4–1.2 (m, 24H, H-3'''–14'''); δ = 0.9 (t, *J* = 8.0 Hz, 3H, H-10'''); δ = 5.7 (bs, 2H, NH₂); 3.9 (s, 3H, –OCH₃).

¹³C NMR (100 MHz, CDCl₃/CD₃OD, ppm): 167.3, 163.4, 159.9, 157.9, 144.7, 143.2, 142.7, 138.0, 137.7, 129.8, 128.3, 120.0, 117.4, 112.2, 104.4, 62.5, 55.6, 31.8, 29.57, 29.56, 29.53, 29.48, 29.41, 29.27, 29.23, 28.95, 26.01, 22.56, 14.0.

Poz. LC-MS/MS *m/z* (%): 489 (1 0 0) [M – ⁷⁹Br/⁸¹Br]⁺, 490 (97) [M – ⁷⁹Br/⁸¹Br + 1]⁺.

Anal. cal. for C₃₁H₄₅BrN₄O (569.63 g/mol): C 65.37, H 7.96, N 9.84, found: C 65.41, H 7.93, N 9.80.

4.2.2.10. 4-[2-Amino-6-(3-methoxyphenyl)pyrimidin-4-yl]-1-pentylpyridinium bromide (6a). Yield: 55%. M.p.: 148–150 °C. Rf: 0.37 (Ethyl acetate/methanol 2:1).

FT-IR (cm⁻¹): 3327, 3207, 3116, 2957, 2933, 2868, 1638, 1599, 1542, 1362, 1264, 1217, 1029.

¹H NMR (400 MHz, CDCl₃/CD₃OD, ppm): δ = 7.8 (s, 1H, H-5); δ = 7.6 (s, 1H, H-2'); δ = 7.1 (d, *J* = 8.0 Hz, 1H, H-4'); δ = 7.4 (t, *J* = 8.0 Hz, 1H, H-5'); δ = 7.7 (d, *J* = 8.0 Hz, 1H, H-6'); δ = 8.8 (d, *J* = 8.0 Hz, 2H, H-2''/6''); δ = 9.2 (d, *J* = 8.0 Hz, 2H, H-3''/5''); δ = 4.7 (t, *J* = 8.0 Hz, 2H, H-1'''); δ = 2.1 (m, 2H, H-2'''); δ = 1.38–1.37 (m, 4H, H-3'''–4'''); δ = 0.9 (t, *J* = 8.0 Hz, 3H, H-5'''); δ = 6.0 (s, 2H, NH₂); 3.9 (s, 3H, –OCH₃).

¹³C NMR (100 MHz, CDCl₃/CD₃OD, ppm): 167.9, 163.8, 160.0, 158.3, 153.1, 144.8, 137.7, 129.9, 125.6, 119.4, 117.3, 112.3, 105.2, 61.8, 55.4, 31.2, 28.0, 21.9, 13.5.

Poz. LC-MS/MS *m/z* (%): 349 (1 0 0) [M – ⁷⁹Br/⁸¹Br]⁺.

Anal. cal. for C₂₁H₂₅BrN₄O (429.36 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.79, H 5.89, N 13.06.

4.2.2.11. 4-[2-Amino-6-(3-methoxyphenyl)pyrimidin-4-yl]-1-decylpyridinium bromide (6b). Yield: 75%. M.p.: 125–127 °C. Rf: 0.40 (Ethyl acetate/methanol 1:1).

FT-IR (cm^{-1}): 3364, 3288, 3181, 3004, 2922, 2851, 1630, 1540, 1449, 1354, 1266, 1042, 835, 777.

^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): $\delta = 7.8$ (s, 1H, H-5); $\delta = 7.7$ (s, 1H, H-2'); $\delta = 7.1$ (d, $J = 8.0$ Hz, 1H, H-4'); $\delta = 7.4$ (t, $J = 8.0$ Hz, 1H, H-5'); $\delta = 7.7$ (d, $J = 8.0$ Hz, 1H, H-6'); $\delta = 8.8$ (d, $J = 8.0$ Hz, 2H, H-2''/6''); $\delta = 9.1$ (d, $J = 8.0$ Hz, 2H, H-3''/5''); $\delta = 4.7$ (t, $J = 8.0$ Hz, 2H, H-1'''); $\delta = 2.0$ (m, 2H, H-2'''); $\delta = 1.4$ – 1.2 (m, 14H, H-3'''-9'''); $\delta = 0.9$ (t, $J = 8.0$ Hz, 3H, H-10'''); $\delta = 6.0$ (s, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): 167.8, 163.7, 160.0, 158.3, 153.0, 144.8, 137.7, 129.9, 125.6, 119.8, 117.3, 112.3, 105.2, 61.8, 55.4, 31.7, 31.5, 29.3, 29.2, 29.0, 28.9, 26.0, 22.5, 28.0, 13.8.

Poz. LC-MS/MS m/z (%): 419 (100) $[\text{M}-^{79}\text{Br}/^{81}\text{Br}]^+$.

Anal. cal. for $\text{C}_{26}\text{H}_{35}\text{BrN}_4\text{O}$ (499.49 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.78, H 5.88, N 13.04.

4.2.2.12. 4-[2-Amino-6-(3-methoxyphenyl)pyrimidin-4-yl]-1-pentadecylpyridinium bromide (**6c**). Yield: 55%. M.p.: 205–207 °C. Rf: 0.48 (Ethyl acetate/methanol 1:1).

FT-IR (cm^{-1}): 3366, 3267, 3179, 2918, 2849, 1639, 1604, 1541, 1359, 1271, 1030, 786.

^1H NMR (400 MHz, CDCl_3 , ppm): $\delta = 7.6$ (s, 1H, H-5); $\delta = 7.6$ (s, 1H, H-2'); $\delta = 7.0$ (d, $J = 8.0$ Hz, 1H, H-4'); $\delta = 7.4$ (t, $J = 8.0$ Hz, 1H, H-5'); $\delta = 7.7$ (d, $J = 8.0$ Hz, 1H, H-6'); $\delta = 8.5$ (d, $J = 8.0$ Hz, 2H, H-2''/6''); $\delta = 9.4$ (d, $J = 8.0$ Hz, 2H, H-3''/5''); $\delta = 4.9$ (t, $J = 8.0$ Hz, 2H, H-1'''); $\delta = 2.0$ (m, 2H, H-2'''); $\delta = 1.3$ – 1.2 (m, 24H, H-3'''-14'''); $\delta = 0.9$ (t, $J = 8.0$ Hz, 3H, H-15'''); $\delta = 5.6$ (s, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, CDCl_3 , ppm): 167.4, 163.5, 160.0, 157.9, 152.5, 145.1, 137.4, 129.9, 125.4, 119.9, 117.7, 112.1, 105.2, 61.8, 55.7, 31.9, 31.7, 29.4, 29.63, 29.62, 29.59, 29.58, 29.54, 29.4, 29.3, 29.0, 26.1, 22.6, 14.1.

Poz. LC-MS/MS m/z (%): 489 (100) $[\text{M}-^{79}\text{Br}/^{81}\text{Br}]^+$, 490 (95) $[\text{M}-^{79}\text{Br}/^{81}\text{Br} + 1]^+$.

Anal. cal. for $\text{C}_{31}\text{H}_{45}\text{BrN}_4\text{O}$ (569.63 g/mol): C 65.37, H 7.96, N 9.84, found: C 65.39, H 7.99, N 9.88.

4.2.2.13. 3-[2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl]-1-pentylpyridinium bromide (**8a**). Yield: 56%. M.p.: 243–245 °C. Rf: 0.48 (Ethyl acetate/methanol 1:2).

FT-IR (cm^{-1}): 3461, 3367, 3188, 2952, 1651, 1572, 1547, 1509, 1369, 1247, 1018, 823.

^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): $\delta = 7.9$ (s, 1H, H-5); $\delta = 8.2$ (d, $J = 8.0$ Hz, 2H, H-2''/6''); $\delta = 7.0$ (d, $J = 8.0$ Hz, 2H, H-3'/5'); $\delta = 9.9$ (s, 1H, H-2''); $\delta = 9.1$ (d, $J = 8.0$ Hz, 1H, H-4''); $\delta = 8.1$ (t, $J = 8.0$ Hz, 1H, 5''); $\delta = 8.9$ (d, $J = 8.0$ Hz, 1H, 6''); $\delta = 4.9$ (t, $J = 8.0$ Hz, 2H, H-1'''); $\delta = 2.1$ (m, 2H, H-2'''); $\delta = 1.40$ – 1.38 (m, 4H, H-3'''-4'''); $\delta = 0.9$ (t, $J = 8.0$ Hz, 3H, H-5'''); $\delta = 5.7$ (s, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): 166.7, 163.3, 162.2, 157.3, 144.5, 143.1, 142.7, 137.9, 129.3, 128.4, 128.3, 114.1, 103.5, 62.3, 55.4, 28.0, 22.0, 13.7.

Poz. LC-MS/MS m/z (%): 349 (100) $[\text{M}-^{79}\text{Br}/^{81}\text{Br}]^+$.

Anal. cal. for $\text{C}_{21}\text{H}_{25}\text{BrN}_4\text{O}$ (429.36 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.80, H 5.84, N 13.08.

4.2.2.14. 3-[2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl]-1-decylpyridinium bromide (**8b**). Yield: 70%. M.p.: 197–199 °C. Rf: 0.40 (Ethyl acetate/methanol 1:2).

FT-IR (cm^{-1}): 3387, 3284, 3190, 3067, 2922, 2853, 1605, 1590, 1505, 1367, 1247, 1173, 1029, 806.

^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): 7.9 (s, 1H, H-5); $\delta = 8.2$ (d, $J = 8.0$ Hz, 2H, H-2''/6''); $\delta = 7.0$ (d, $J = 8.0$ Hz, 2H, H-3'/5'); $\delta = 10.0$ (s, 1H, H-2''); $\delta = 9.1$ (d, $J = 8.0$ Hz, 1H, H-4''); $\delta = 8.1$ (t, $J = 8.0$ Hz, 1H, 5''); $\delta = 9.0$ (d, $J = 8.0$ Hz, 1H, 6''); $\delta = 4.9$ (t, $J = 8.0$ Hz, 2H, H-1'''); $\delta = 2.0$ (m, 2H, H-2'''); $\delta = 1.38$ – 1.23 (m, 14H,

H-3'''-9'''); $\delta = 0.8$ (t, $J = 8.0$ Hz, 3H, H-10'''); $\delta = 5.6$ (s, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): 167.0, 163.4, 162.3, 157.4, 144.5, 143.1, 142.7, 138.1, 129.2, 128.5, 128.3, 114.1, 103.6, 62.4, 55.4, 31.8, 31.7, 29.3, 29.2, 29.1, 28.9, 26.0, 22.5, 13.9.

Poz. LC-MS/MS m/z (%): 419 (100) $[\text{M}-^{79}\text{Br}/^{81}\text{Br}]^+$, 420 (88) $[\text{M}-^{79}\text{Br}/^{81}\text{Br} + 1]^+$.

Anal. cal. for $\text{C}_{26}\text{H}_{35}\text{BrN}_4\text{O}$ (499.49 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.78, H 5.90, N 13.10.

4.2.2.15. 3-[2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl]-1-pentadecylpyridinium bromide (**8c**). Yield: 54%. M.p.: 210–212 °C. Rf: 0.35 (Ethyl acetate/methanol 1:2).

FT-IR (cm^{-1}): 3387, 3284, 3187, 3073, 2916, 2850, 1605, 1546, 1505, 1368, 1246, 1172, 1029, 807.

^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): $\delta = 8.0$ (s, 1H, H-5); $\delta = 8.3$ (d, $J = 8.0$ Hz, 2H, H-2''/6''); $\delta = 7.0$ (d, $J = 8.0$ Hz, 2H, H-3'/5'); $\delta = 10.1$ (s, 1H, H-2''); $\delta = 9.2$ (d, $J = 8.0$ Hz, 1H, H-4''); $\delta = 8.1$ (t, $J = 8.0$ Hz, 1H, 5''); $\delta = 9.0$ (d, $J = 8.0$ Hz, 1H, 6''); $\delta = 5.0$ (t, $J = 8.0$ Hz, 2H, H-1'''); $\delta = 2.1$ (m, 2H, H-2'''); $\delta = 1.38$ – 1.23 (m, 24H, H-3'''-14'''); $\delta = 0.9$ (t, $J = 8.0$ Hz, 3H, H-15'''); $\delta = 5.4$ (s, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): 167.0, 163.4, 162.3, 157.4, 144.5, 143.1, 142.7, 138.1, 129.2, 128.5, 128.3, 114.1, 103.6, 62.4, 55.3, 31.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 28.7, 28.1, 26.0, 22.7, 14.0.

Poz. LC-MS/MS m/z (%): 489 (100) $[\text{M}-^{79}\text{Br}/^{81}\text{Br}]^+$, 490 (96) $[\text{M}-^{79}\text{Br}/^{81}\text{Br} + 1]^+$.

Anal. cal. for $\text{C}_{31}\text{H}_{45}\text{BrN}_4\text{O}$ (569.63 g/mol): C 65.37, H 7.96, N 9.84, found: C 65.42, H 7.94, N 9.88.

4.2.2.16. 4-[2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl]-1-pentylpyridinium bromide (**9a**). Yield: 62%. M.p.: 187–189 °C. Rf: 0.48 (Ethyl acetate/methanol 1:1).

FT-IR (cm^{-1}): 3421, 3274, 3187, 2927, 1580, 1537, 1510, 1362, 1240, 1173, 1024, 812, 785.

^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): $\delta = 7.7$ (s, 1H, H-5); $\delta = 8.1$ (d, $J = 8.0$ Hz, 2H, H-2''/6''); $\delta = 7.0$ (d, $J = 8.0$ Hz, 2H, H-3'/5'); $\delta = 8.7$ (d, $J = 8.0$ Hz, 2H, H-2''/6''); $\delta = 9.1$ (d, $J = 8.0$ Hz, 2H, H-3''/5''); $\delta = 4.7$ (t, $J = 8.0$ Hz, 2H, H-1'''); $\delta = 2.0$ (m, 2H, H-2'''); $\delta = 1.4$ – 1.3 (m, 4H, H-3'''-4'''); $\delta = 0.9$ (t, $J = 8.0$ Hz, 3H, H-5'''); $\delta = 5.7$ (s, 2H, NH_2); 3.8 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): 167.6, 163.7, 162.4, 157.9, 153.3, 144.8, 129.1, 128.6, 125.4, 114.3, 104.6, 61.9, 55.4, 31.2, 28.0, 22.0, 13.6.

Poz. LC-MS/MS m/z (%): 349 (100) $[\text{M}-^{79}\text{Br}/^{81}\text{Br}]^+$.

Anal. cal. for $\text{C}_{21}\text{H}_{25}\text{BrN}_4\text{O}$ (429.36 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.80, H 5.88, N 13.08.

4.2.2.17. 4-[2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl]-1-decylpyridinium bromide (**9b**). Yield: 80%. M.p.: 217–219 °C. Rf: 0.50 (Ethyl acetate/methanol 1:2).

FT-IR (cm^{-1}): 3452, 3325, 3210, 2925, 2852, 1621, 1545, 1512, 1361, 1246, 1171, 1024, 821.

^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): $\delta = 7.7$ (s, 1H, H-5); $\delta = 8.1$ (d, $J = 8.0$ Hz, 2H, H-2''/6''); $\delta = 7.0$ (d, $J = 8.0$ Hz, 2H, H-3'/5'); $\delta = 8.7$ (d, $J = 8.0$ Hz, 2H, H-2''/6''); $\delta = 9.1$ (d, $J = 6.4$ Hz, 2H, H-3''/5''); $\delta = 4.7$ (t, $J = 8.0$ Hz, 2H, H-1'''); $\delta = 2.0$ (m, 2H, H-2'''); $\delta = 1.3$ – 1.2 (m, 14H, H-3'''-9'''); $\delta = 0.8$ (t, $J = 8.0$ Hz, 3H, H-5'''); $\delta = 5.7$ (s, 2H, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): 167.5, 163.7, 162.4, 157.9, 153.3, 144.7, 129.0, 128.5, 125.5, 114.2, 104.5, 61.8, 55.3, 31.7, 31.5, 29.3, 29.2, 29.1, 28.9, 26.0, 22.5, 13.9.

Poz. LC-MS/MS m/z (%): 420 (100) $[\text{M}-^{79}\text{Br}/^{81}\text{Br} + 1]^+$.

Anal. cal. for $\text{C}_{26}\text{H}_{35}\text{BrN}_4\text{O}$ (499.49 g/mol): C 58.75, H 5.87, N 13.05, found: C 58.77, H 5.84, N 13.07.

4.2.2.18. 4-[2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl]-1-pentadecylpyridinium bromide (**9c**). Yield: 55%. M.p.: 181–183 °C. Rf: 0.48 (Ethyl acetate/methanol 1:2).

FT-IR (cm^{-1}): 3425, 3281, 3193, 2916, 2849, 1606, 1539, 1511, 1364, 1242, 1176, 1026, 812.

^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): $\delta = 7.5$ (s, 1H, H-5); $\delta = 8.1$ (d, $J = 8.0$, 2H, H-2'/6'); $\delta = 7.0$ (d, $J = 8.0$ Hz, 2H, H-3'/5'); $\delta = 8.7$ (d, $J = 6.0$ Hz, 2H, H-2''/6''); $\delta = 9.1$ (d, $J = 6.0$ Hz, 2H, H-3''/5''); $\delta = 4.7$ (t, $J = 8.0$ Hz, 2H, H-1'''); $\delta = 2.0$ (m, 2H, H-2'''); $\delta = 1.3$ – 1.2 (m, 24H, H-3'''–14'''); $\delta = 0.8$ (t, $J = 8.0$ Hz, 3H, H-15'''); $\delta = 5.7$ (s, NH_2); 3.9 (s, 3H, $-\text{OCH}_3$).

^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$, ppm): 167.7, 163.8, 162.4, 158.0, 153.5, 144.8, 129.0, 128.7, 125.6, 114.3, 104.6, 61.9, 55.4, 31.8, 31.6, 29.58, 29.55, 29.54, 29.49, 29.40, 29.27, 29.26, 29.25, 28.92, 26.05, 22.57, 13.9.

Poz. LC-MS/MS m/z (%): 489 (1 0 0) $[\text{M}^{-79}\text{Br}^{81}\text{Br}]^+$, 490 (94) $[\text{M}^{-79}\text{Br}^{81}\text{Br} + 1]^+$.

Anal. cal. for $\text{C}_{31}\text{H}_{45}\text{BrN}_4\text{O}$ (569.63 g/mol): C 65.37, H 7.96, N 9.84, found: C 65.42, H 8.0, N 9.86.

4.2.3. Pharmacology

4.2.3.1. *Preparation of cell culture.* The anticancer potential of the compounds were investigated on cancerous HeLa (ATCC® CCL2™), HT29 (ATCC® HTB38™), MCF7 (ATCC® HTB22™), A549 (ATCC® CCL185™), C6 (Rat brain glioma, ATCC® CCL-107™), and Hep3B (ATCC® HB8064™) and normal FL cells (ATCC® CCL62™). The cell lines were cultured in a cell medium (Dulbecco's modified eagle's or RPMI 1640) enriched with 10% (v/v) fetal bovine serum and 2% (v/v) Penicillin-Streptomycin (10,000 U/mL). First, old medium was removed out of the flask while cells had reached approximately 80% confluence. Next, cells were taken from the flasks surface using trypsin-EDTA solution and then subjected to centrifugation. Following, the cell pellet was suspended with fresh media and was inoculated into wells.

4.2.3.2. *Cell proliferation assay (MTT assay).* A cell suspension containing approximately 1×10^4 cells in 100 μL was seeded into the wells of 96-well culture plates. The cells were treated with the compounds and control drug, cis-platin and 5 fluorouracil (5FU), dissolved in sterile DMSO (max 0.5% of DMSO) at final concentrations of 1.96, 3.91, 7.81, 15.625, 31.25, 62.5, 125, and 250 $\mu\text{g}/\text{mL}$ at 37 °C with 5% CO_2 for overnight. The final volume of the wells was set to 200 μL by medium. Cell proliferation assay was evaluated by MTT (yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) methods. Briefly, An MTT stock solution (5 mg of MTT/mL of distilled water) was filtered and kept for at -20 °C until use. The cells were exposed to a MTT reagent (consisting of one parts of MTT stock solutions and nine parts of fresh RPMI 1640 without phenol red) for 4 h to form MTT formazan dye followed by the dye dissolved in DMSO with Sorenson's buffer for 30 min at room temperature and then the plate was measured at 560 nm, with 690 nm as a reference interval, using a microplate reader. Each experiment was repeated at least three times for each cell line.

4.2.3.3. *Cytotoxic activity assay.* The cytotoxicity of the compounds, cisplatin and 5 fluorouracil on cells was determined through a Lactate Dehydrogenase Assay Kit according to the manufacturer's instructions. Approximately 5×10^3 cells in 100 μL were placed into 96-well plates as triplicates and treated with 25, 50, 75, and 100 $\mu\text{g}/\text{mL}$ concentrations of test compounds at 37 °C with 5% CO_2 for 24 h. LDH activity was obtained by determining absorbance at 492–630 nm using a microplate reader. The cytotoxicity assay results were noted as the percent cytotoxicity according to the following formula: % Cytotoxicity = $[(\text{Experimental Value} - \text{Low Control}/\text{High Control} - \text{Low Control}) \times 100]$.

4.2.3.4. *BrdU cell proliferation assay (BCPA).* A Cell suspension

containing 5×10^3 cells in 100 μL was pipeted into wells of 96-well cell culture plates (COSTAR, Corning, USA). Test compounds were dissolved in sterile DMSO. DMSO amount was adjusted to 0.5%. The cells were treated with test compounds at final concentrations of 1000, 2000, 4000, 6000, 8000, 10000, 15000, and 20000 ng/mL . Cell controls and solvent controls were treated with supplemented DMEM and sterile DMSO respectively. The final volume of the wells was adjusted to 200 μL by supplemented DMEM. The cells then were incubated at 37 °C with 5% CO_2 for overnight. The antiproliferative activity of the compounds was determined using BrdU Cell proliferation ELISA Kit (Roche, USA), a calorimetric immunoassay based on BrdU incorporation into the cellular DNA, according to manufacturer's protocol. Briefly, cells were exposed to BrdU labeling reagent for 4 h followed by fixation in FixDenat solution for 30 min at room temperature. Then, cells were cultured with 1:100 diluted anti-BrdU-POD for 1.30 h at room temperature, substrate solution was added to each well and BrdU incorporation was measured at 450–650 nm using a microplate reader (Rayto, China). Each experiment was repeated at least three times for each cell line.

4.2.3.5. *Microdilution assay.* MIC values of the compounds against bacterial strains were determined on the basis of a micro-well dilution method. To determine the minimal inhibitory concentration (MIC) values, inocula of bacteria were prepared using 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Each substance dissolved in dimethyl sulfoxide (DMSO) and serial twofold dilutions were made in a concentration range from 7.81 to 1000 $\mu\text{g}/\text{mL}$ in microplate wells containing nutrient broth. Growth of microorganisms was determined visually after incubation for 24 h at 35 °C. The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC.

4.2.3.6. *DNA binding studies.* To find the interaction of the compounds with calf thymus DNA and to calculate the binding constants (K_b), UV-Visible absorption spectroscopy technique was used. A 2.5 mg calf thymus DNA was dissolved in 10.0 mL Tris-HCl buffer (20 mM Tris-HCl, 20 mM NaCl, pH 7.0) and stable during one week in the refrigerator. The concentration of calf thymus DNA was obtained spectrophotometrically with help of ϵ value of $6600 \text{ M}^{-1} \text{ cm}^{-1}$ at 260 nm. After dissolving the calf thymus DNA fibers in Tris-HCl buffer, the purity of this solution was checked from the absorbance ratio A260/A280. The calf thymus DNA solution at A260/A280 ratio was equal to 1.87, implying that the DNA was pure enough. These compounds were diluted with Tris-HCl buffer to obtain 25 μM concentrations. Test compounds in the solutions were incubated at room temperature for nearly 30 min before the process. The UV-visible spectral studies were performed in a mixed solvent system (1/9 DMSO/Tris-HCl buffer) using eight points and the fine structure is observed by UV-visible absorption. The UV absorption titrations were conducted by keeping the concentration of these compounds fixed while varying the CT-DNA concentrations (6.5–800 μM). Absorption spectra were recorded by using 1-cm-path quartz cuvettes at room temperature. To evaluate the interaction of the compounds with BSA was also used a UV-Visible absorption spectroscopy technique. A 2.5 mg BSA was dissolved in 10.0 mL in Tris-HCl buffer (5 mM Tris-HCl, 10 mM NaCl at pH 7.4) and stored in the refrigerator. The UV-Visible absorption spectra of the BSA solutions (6.5–800 μM) in the presence of a conserved concentration of the compounds (25 μM) were scanned in the wavelength range from 300 to 550 nm.

4.2.3.7. *Calculation of IC_{50} and three dose response parameters.* IC_{50} value is a concentration that inhibits half of the cells in vitro. The half maximal inhibitory concentration (IC_{50}) of the test and control compounds was calculated using XLfit5 or Excel spreadsheet and represent in μM at 95% confidence intervals. The proliferation assay results were expressed as the percent inhibition according to the

following formula: % Inhibition = $[1 - (\text{Absorbance of Treatments} / \text{Absorbance of DMSO}) \times 100]$. Three dose response parameters (GI_{50} , TGI, LC_{50}) were calculated according to the following formulas using the absorbance measurements of time zero (Tz), control growth (C), and test growth in the presence of drug (Ti). Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net growth increase in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from $Ti = Tz$. The LC_{50} indicating a net loss of cells following treatment was calculated from $[(Ti-Tz)/Tz] \times 100 = -50$.

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