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New Thiazolopyrimidine as anticancer agents: Synthesis, biological evaluation, DNA binding, Molecular modeling and ADMET study.

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Abstract

In the present study, new series of thiazolopyrimidine derivatives was synthesized as purine analogs. The structures of the products were confirmed through spectroscopic techniques such as NMR and mass spectrometry. In addition, the synthesized compounds were evaluated as antitumor active agent through NCI screening protocol against 60 different cell lines under 9 different panels. Furthermore, DNA binding activity of the compounds was also evaluated. The results revealed that compound **35** proved to be the most active member of the tested series and it is promoted to the 5-dose testing where it gives GI_{50}, TGI and LC_{50} values of 1.07, 6.61, 34.7 μM respectively. Furthermore, it also proved to have a good DNA binding activity with value that is comparable with that produced by doxorubicin which was used as positive standard. In addition, compound **27** was proved to be the most active DNA binding agent with binding affinity **28.38 ± 1.1**. The pharmacokinetic properties were also calculated**.** Molecular docking studies suggested binding mode of compounds **27** and **35** to DNA minor groove *via* hydrogen bonding interaction**.** The anticancer activity of compounds **27** and **35** may be attributed to DNA binding.

Keywords: Synthesis, thiazolopyrimidine, purine, DNA-binding, antitumor, ADMET, Molecular Docking

Nitrogenous bases are heterocyclic scaffolds that play several important roles in biology. They are the major skeleton of many vital biomolecules. Both pyrimidine and purine pharmacophores are the building blocks that form the backbone of genetic information carrying molecules such as DNA and RNA. Also, they are involved in other biological processes such as cell signaling. This caused a considerable utilization of them in the drug designing process of several chemotherapeutic agents, cardiovascular as well as agrochemical and veterinary drugs [1]. Many purine and pyrimidine analogs have versatile biological activity including anti-inflammatory and analgesic, antimicrobial, anti-avian influenza virus (H5N1), against herpes simplex virus type-1 (HSV-1) and hepatitis-A virus (HAV), serotonin 5-HT_6 receptor antagonist, anti-arrhythmic agents, etc. [2,3].

The pyrimidine core constructs the major skeleton of many well-known medications such as fluorouracil, etravirine, risperidone, iclaprim, avanafil and rosuvastatin. On the other hand, many drugs are modified purines such as cladribine, tioguanine, clofarabine, mercaptopurine, fludarabine and nelarabine. These represent a broad number of medication classes resulting in drawing the attention to such class of compounds as candidates for design and development of new biologically active compounds [4-11].

Taking a closer look to the anticancer activity of both purine and pyrimidine analogs, these skeletons are involved in many antimetabolites that were used for decades as chemotherapeutic drugs, for example, 6-mecaptopurine (Lukerine), thioguanine, azathioprine (Imuran) and azaguanine (Guanazole) represents examples of purine bases-containing drugs while 5-fluorouracil (Fluroplex), cytarabine (Cytosar) represents the most known pyrimidine containing chemotherapeutic agents [12]. These drugs mainly act through the inhibition of many enzymes involved in both salvage and *de novo* synthesis of purine and pyrimidine bases such as phosphoribosyl transferase, aminotransferase, adenyl succinate synthase, adenyl succinate lyase and etc….. [12]. In many cases, the purine metabolites can block certain enzymatic pathways or they can mimic the reactivity of the natural purines as substrates to be metabolized to their corresponding ribonucleotide and deoxyribonucleotide triphosphates and subsequently incorporated into DNA and RNA. As a result, interference with nucleic acid synthesis and function causes multiple types of toxicities [5,6].

Thiazolopyrimidines are purine isosteres which are involved in many drug skeletons, (Fig. 1 A) it was revealed that thiazolo[*3,2-a*]pyrimidine (Fig. 1 B) is an efficient scaffold in the field of anticancer research as well as chemotherapeutic agents. Their activity was proved to be attributed to the intercalation with the DNA that is the major target in fighting the cancer cells [13,14]. Most of the well-known agents which are successfully used in clinical treatment are small molecules that are able to interact with nucleic acid mostly through one of these modes: intercalation, groove binding or electrostatic interactions [15, 16]. In view of the mentioned data and in continuation of our work [17-20], we synthesized new thiazolo[3,2-*a*]pyrimidine analogs in a trial to discover more selective and potent antitumor agents (Fig.2). through the designing process of the target compounds many features that revealed to be important in the synthesized structure were preserved. These results were obtained from the previous work already published. From these features, the methoxy groups that are known to enhance the antitumor potency [21, 22]. The second feature is represented by the ethyl carboxylate moiety that improve the activity of compounds containing it [17]. *In-vitro* anticancer screening of the synthesized compounds was conducted by the NCI protocol on 60 cell lines cancer subpanels namely [23-25]. In addition, DNA binding assay of the synthesized compounds was also conducted as a trial

for detection of the mechanism of action of the most potent compounds of the synthesized series [26]. In silico study describing the properties of the target compounds and DNA binding activities were also carried out. The structures of the synthesized compounds were confirmed by different spectroscopic techniques involving H^1NMR, C^{13}NMR and mass spectroscopy. In addition, the products were evaluated as antitumor agent through NCI screening protocol against 60 different cell lines under 9 different panels: Leukemia, Renal cancer, CNS cancer, Prostate cancer, Non-small lung cancer, Colon cancer, Ovarian cancer, Breast cancer and Melanoma. Furthermore, DNA binding activity of the compounds was also evaluated.

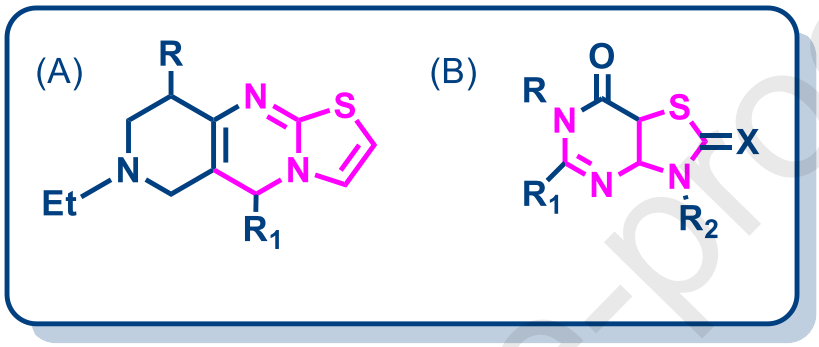


Figure 1 Structures of important reference anticancer agents

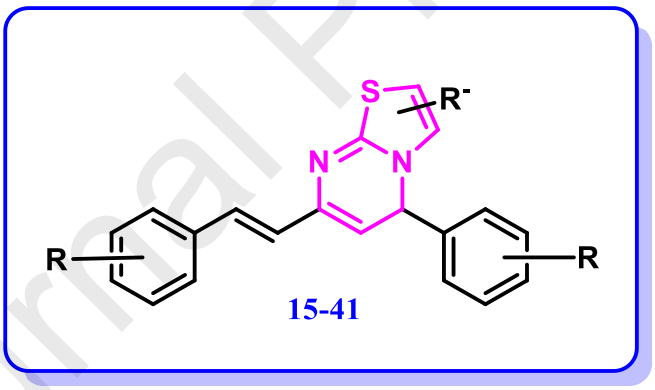
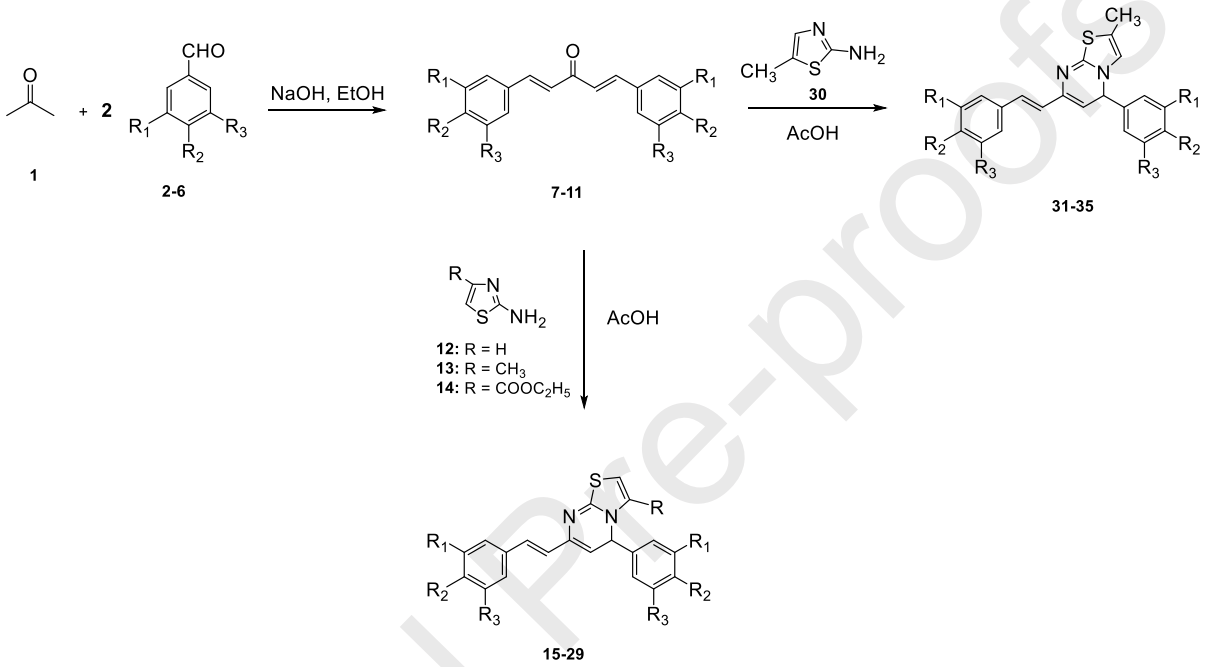


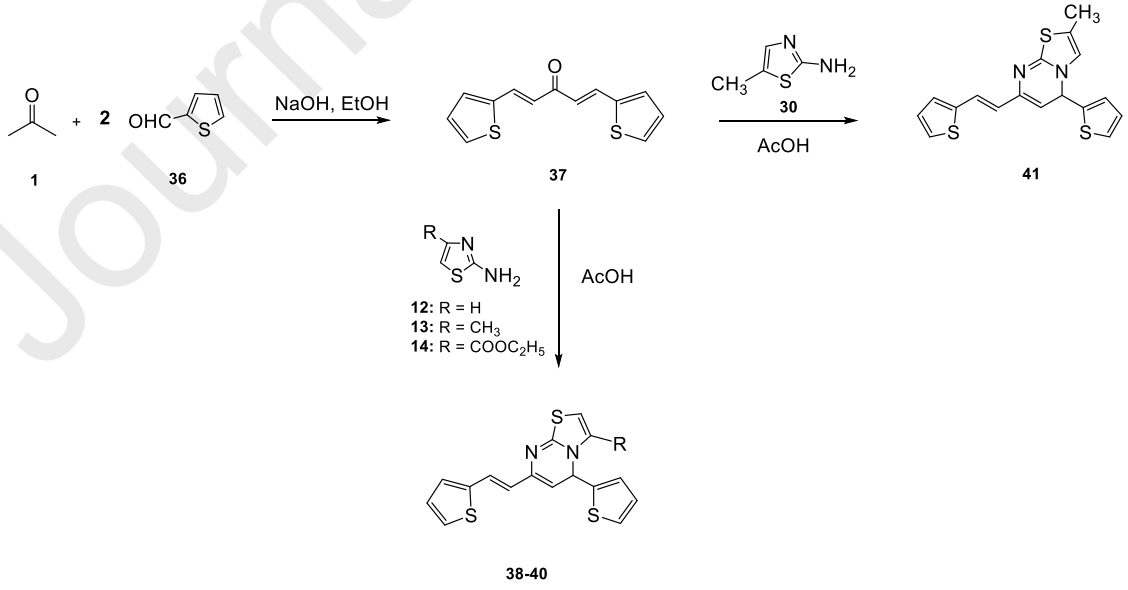
Figure 2 Structures of the designed synthesized compounds

The synthetic strategy for the synthesis of the target products **15-29, 31-35** and **38-41** is illustrated in schemes 1 and 2. The intermediate compounds **7-11** were prepared through the interaction of pure acetone and substituted benzaldehyde in 10 % alcoholic NaOH to yield the diarylidene derivatives [18-20]. The latter were further reacted with either 2-aminothiazole **(12)**, 2-amino-4-methylthiazole **(13)**, Ethyl 2-aminothiazole-4-carboxylate **(14)**, 2-amino-5-methylthiazole **(30)** to afford the target compounds **15-29** and **31-35**.

The same strategy was adopted for the synthesis of compounds **38-41**, as the substituted benzaldehydes were replaced by 2-thiophene carboxaldehyde to yield the intermediate **37** which is further reacted with substituted thiazoles **12-14** and **30** to give the target compounds **38-41** (Table 1). The spectral data confirmed the structures of the target compounds. For example, the appearance of the methyl and the ethyl ester group or even the appearance of additional aromatic protons of the thiazole moiety in the HNMR spectrum indicates the formation of the products in addition to the molecular weight in the mass spectrometric charts.

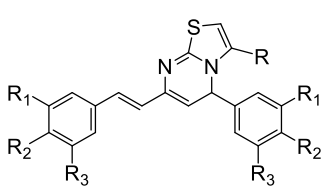


Scheme 1: Synthesis of compounds 15-29 and 31-35

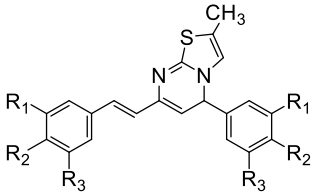


Scheme 2: Synthesis of compounds 38-41

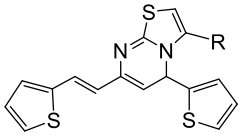
Table 1: Physicochemical Properties of the target compounds **15-29**, **31-35** and **38-41**



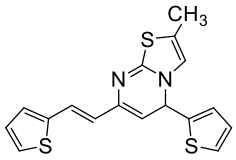
15-29



31-35



38-40



41

Comp. no	R	R^1	R^2	R^3	m.p.	Yield %	Comp. no	R	R^1	R^2	R^3	m.p.	Yield %
15	**H**	**H**	**Cl**	**H**	**140-3**	**55**	**27**	**$COOC_2H_5$**	**H**	**OCH_3**	**H**	**188-90**	**59**
16	**H**	**H**	**CH_3**	**H**	**153-6**	**58**	**28**	**$COOC_2H_5$**	**OCH_3**	**OCH_3**	**H**	**200-3**	**66**
17	**H**	**H**	**OCH_3**	**H**	**166-9**	**61**	**29**	**$COOC_2H_5$**	**OCH_3**	**OCH_3**	**OCH_3**	**176-80**	**61**
18	**H**	**OCH_3**	**OCH_3**	**H**	**175-7**	**55**	**31**	**-**	**H**	**Cl**	**H**	**144-5**	**55**
19	**H**	**OCH_3**	**OCH_3**	**OCH_3**	**204-9**	**62**	**32**	**-**	**H**	**CH_3**	**H**	**121-5**	**52**
20	**CH_3**	**H**	**Cl**	**H**	**151-4**	**43**	**33**	**-**	**H**	**OCH_3**	**H**	**157-62**	**54**
21	**CH_3**	**H**	**CH_3**	**H**	**180-3**	**59**	**34**	**-**	**OCH_3**	**OCH_3**	**H**	**134-7**	**49**
22	**CH_3**	**H**	**OCH_3**	**H**	**137-9**	**61**	**35**	**-**	**OCH_3**	**OCH_3**	**OCH_3**	**191-5**	**42**
23	**CH_3**	**OCH_3**	**OCH_3**	**H**	**126-9**	**46**	**38**	**H**	**-**	**-**	**-**	**190-3**	**55**
24	**CH_3**	**OCH_3**	**OCH_3**	**OCH_3**	**194-8**	**52**	**39**	**CH_3**	**-**	**-**	**-**	**215-7**	**49**
25	**$COOC_2H_5$**	**H**	**Cl**	**H**	**171-5**	**76**	**40**	**$COOC_2H_5$**	**-**	**-**	**-**	**164-8**	**44**
26	**$COOC_2H_5$**	**H**	**CH_3**	**H**	**130-4**	**65**	**41**	**-**	**-**	**-**	**-**	**220-4**	**42**

Fifteen of the synthesized compounds were screened using the NCI's disease-oriented human cell lines screening assay for their *in-vitro* antitumor activity using single dose (10 µM) of the test compounds against 60 cell lines representing nine tumor subpanels namely; leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cells [23-25]. Compounds that proved to be highly active were promoted to the five-dose screen. The data obtained were presented in form of percentage growth inhibition (GI %). The obtained results of the tested thiazolo [3,2-*a*]pyrimidine analogs showed distinctive potential pattern of selectivity, as well as broad-spectrum antitumor activity (Tables **2** and **3**). Doxorubicin was used as a positive control. As shown in tables **2** and **3**, it was observed that most of the synthesized compounds proved to be inactive against almost all the tested cell lines, having GI% not exceeding 20% for most of the tested compounds. On the other hand, compounds **29** and **35** proved to be the active members between the synthesized products. Compound **29** showed activity against most of the tested cell lines with GI% ranging from 12.0- 69.3 %, in addition its activity was observed more against the Leukemia cell lines in which it exerts its high GI% 69.3 value. Furthermore, concerning the most active compound **35**, it showed lethal effect on almost all the cell lines and its least GI% was achieved against CNS cancer (SNB-75), these results promoted this compound to the 5-dose screening in which 5 different concentrations of the compound under investigation are prepared and tested against the 9 different types of cancer cells.

It was observed that the GI_{50} of compound **35** was about 10 folds more than that of the reference compound doxorubicin, and about the TGI is 3 folds more than the reference drug while the LC_{50} is nearly equal to that of doxorubicin. By taking a closer look to each cell line individually, in general, most of the target compounds showed inactivity against the majority of the cell lines used for screening, but for example, leukemia cell lines, compounds **25, 29, 32** and **35** showed activity against K-562 and MOLT-4. The Non-small cell lung cancer HOP-92 and NCI-H522 were also found to be sensitive to some of the tested compounds such as **28, 29** and **35**. Regarding CNS cancer cell line SNB-75, it was observed that it is sensitive to a number of compounds with GI% inhibition ranging from 12.3-75.6 %. Finally, renal cancer cell lines were also sensitive to some of the test compounds **18, 23, 25, 28, 29, 32** and **35**.

Table 2: Percentage growth inhibition (GI%) of *in vitro* subpanel tumor cell lines at 10 μM concentration of the selected compounds

Subpanel tumor cell lines	% Growth Inhibition (GI %)[a]												
	17	**18**	**23**	**24**	**25**	**28**	**29**	**31**	**32**	**35**	**38**	**39**	**Doxorubicin**
Leukemia													
CCRF-CEM	-	-	-	-	-	-	69.3	-	-	L	-	-	**L**
HL-60(TB)	-	-	-	-	-	-	-	-	-	L	-	-	**L**
K-562	-	-	-	-	12.4	-	35.7	-	-	97.8	-	-	97.9
MOLT-4	-	-	-	-	16.7	-	25.1	-	12.6	L	-	-	**L**
RPMI-8226	-	-	-	-	-	-	18.0	-	-	L	-	-	**L**
SR	-	13.8	-	-	-	-	59.0	-	-	L	-	-	**L**
Non-small cell lung cancer													
A549/ATCC	-	-	-	-	-	-	-	-	-	L	-	-	**L**
HOP-62	-	-	-	-	-	-	-	-	-	L	-	-	93.1
HOP-92	10.1	-	18.0	-		39.5	-	-	-	L	-	-	**L**
NCI-H226	-	-	-	-	-	-	-	-	-	97.3	-	-	**L**
NCI-H23	-	-	-	-	-	-	-	-	-	L	-	-	66.7
NCI-322M	-	-	-	-	-	-	-	-	-	86.4	-	-	86.1
NCI-H460	-	-	-	-	-	-	-	-	-	L	-	-	99.4
NCI-H522	-	-	-	14.5	-	12.0	47.8	-	10.9	L	-	13.3	**L**
Colon cancer													
COLO 205	-	-	-	-	-	-	-	-	-	L	-	-	**L**
HCC-2998	-	-	-	-	-	-	-	-	-	L	-	-	**L**
HCT-116	-	-	-	-	-	-	44.4	-	-	L	-	-	97.2
HCT-15	-	-	-	-	-	-	35.0	-	-	L	-	-	84.8
HT29	-	-	-	-	-	-	13.0	-	-	L	-	-	95.3
KM12	-	-	-	-	-	-	22.7	-	-	L	-	-	95.8
SW-620	-	-	-	-	-	-	13.4	-	-	L	-	-	98.1
CNS cancer													
SF-268	-	-	-	-	-	-	-	-	-	L	-	-	**L**
SF-295	-	-	-	-	-	-	-	-	-	L	-	-	**L**
SF-539	-	-	-	-	-	-	-	-	-	L	-	-	**L**
SNB-19	-	-	-	-	-	-	-	-	-	66.5	-	-	**L**
SNB-75	-	-	-	12.5	-	25.3	18.4	17.3	13.7	75.6	12.3	-	**L**
U251	-	-	-	-	14.6	-	-	-	-	L	-	-	90.8
Melanoma													
LOX IMVI	-	-	-	-	-	-	29.7	-	-	L	-	-	94.3
MALME-3M	-	-	-	-	-	-	-	-	-	L	-	-	**L**
M14	-	-	-	-	-	-	-	-	-	L	-	-	**L**
MDA-MB-435	-	-	-	-	-	-	-	-	-	L	-	-	**L**
SK-MEL-2	-	-	-	-	-	-	-	-	-	L	-	-	**L**
SK-MEL-28	-	-	-	-	-	-	-	-	-	L	-	-	**L**
SK-MEL-5	-	-	-	-	-	-	12.0	-	-	L	-	-	**L**
UACC-257	11.9	12.8		-	-	-	17.2	-	-	L	-	-	96.5
UACC-62	-	-	-	-	-	-	18.0	-	-	L	-	-	**L**

Ovarian cancer													
IGORV1	-	-	-	-	-	-	-	-	-	L	-	-	**L**
OVCAR-4	-	-	-	-	-	-	12.5	-	-	88.4	-	-	85.9
OVCAR-5	-	-	-	-	-	-	-	-	-	L	-	-	**L**
OVCAR-8	-	-	-	-	-	-	32.0	-	-	91.9	-	-	**L**
NCI/ADR-RES	-	-	-	-	-	-	-	-	-	80.6	-	-	95.1
SK-OV-3	-	-	-	-	-	-	-	-	-	L	-	-	86.2
Renal cancer													
786-0	-	-	-	-	-	-	-	-	-	L	-	-	95.5
A498	-	25.6	17.6	-	11.8	-	-	12.3	-	L	-	-	**L**
ACHN	-	-	-	-	-	12.8	-	-	-	L	-	-	**L**
CAKI-1	-	-	-	-	-	-	-	-	-	L	-	-	**L**
RXF 393	-	-	-	-	-	-	-	-	-	L	-	-	**L**
SN12C	-	-	-	-	-	-	-	-	-	L	-	-	88.0
TK-10	-	-	-	-	-	-	-	-	-	L	-	-	74.6
UO-31	-	11.3	11.7	-	-	20.2	13.7	-	19.1	L	-	-	96.0
Prostate cancer													
PC-3	17.2		-	-	-	11.3	21.1	-	-	L	-	-	98.7
DU-145	-	-	-	-	-	-	-	-	-	L	-	-	86.8
Breast cancer													
MCF7	-	-	-	-	-	-	28.9	-	-	L	-	-	96.6
MDA-MB-231/ATCC	-	-	-	-	-	-	14.5	-	-	L	-	-	96.2
HS 578T	-	-	-	-	-	-	-	-	-	98.0	-	-	**L**
BT-549	-	-	-	-	-	-	-	-	-	L	-	-	**L**
T-47D	-	-	-	-	-	-	-	-	-	92.0	-	-	**L**
MDA-MB-468	-	-	-	-	-	-	-	-	11.3	L	-	-	**L**

[a] -, GI < 10%; nt, not tested; L, compound proved lethal to the cancer cell line.

Table 3: Compound 35 Median growth inhibitory (GI_{50}, μM), Total growth inhibitory (TGI, μM) and Median lethal (LC_{50}, μM) concentrations of *in vitro* subpanel tumor cell lines

Compound	Activity	I	II	III	IV	V	VI	VII	VIII	IX	MG-MID[a]
35	GI_{50}	0.43	1.94	0.92	1.85	0.99	1.75	1.49	1.69	1.39	1.07
	TGI	61.93	4.81	3.62	10.15	3.37	33.9	4.38	52.9	17.90	6.61
	LC_{50}	b	62.85	36.0	54.25	18.23	76.45	54.73	b	68.71	34.7
Doxorubicin	GI_{50}										0.15
	TGI										2.48
	LC_{50}										30.5

I, leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer. [a]Full panel mean-graph midpoint (μM). b Compounds showed values > 100 μM. nt, not tested.

DNA represents an important target for many drugs currently in clinical use or in advanced clinical trials. It was proved that small molecules can act as potent antimicrobials *via* DNA intercalation. Methyl green was used in Methyl green-DNA Displacement assay to reversibly bind to DNA, and the colored complex is stable at neutral pH, whereas free methyl green fades at this pH value. Incubation for 24 h, in the buffer used for displacement reactions in this study, results in virtually a complete loss of methyl green absorbance. A colorimetric assay was used to measure the displacement of methyl green from DNA by compounds with ability to bind to DNA [26]. From the obtained results, it was observed that compound **27** is the most active one among the synthesized compounds; it had IC_{50} value less than that obtained by the reference drug Doxorubicin. Other compounds proved to be active

such as compounds **28, 18, 26** and **38** with IC_{50} values of **29.86 ± 1.3**, **30.05 ± 1.6**, **31.49 ± 1.8** and **33.67 ± 1.8** respectively. On the other hand, compound **35** that represents the most active product in the antitumor activity had IC_{50} value **36.13 ± 1.9** which is very close to the standard, giving indication that its mechanism of action can be due to its DNA binding action (Table 4).

Table 4: DNA/methyl green colorimetric assay of the DNA-binding compounds

No.	DNA-active compound	DNA/methyl green (IC50 µg/ml)	No.	DNA-active compound	DNA/methyl green (IC50 µg/ml)
*	DOX	31.27±1.8			
1	15	56.33 ± 2.9	13	27	28.38 ± 1.1
2	16	62.74 ± 3.2	14	28	29.86 ± 1.3
3	17	47.75 ± 2.6	15	29	43.92 ± 2.3
4	18	30.05 ± 1.6	16	31	45.18 ± 2.5
5	19	55.42 ± 3.6	17	32	87.57 ± 4.3
6	20	50.91 ± 2.7	18	33	41.37 ± 2.1
7	21	83.41 ± 4.1	19	34	58.32 ± 3.0
8	22	42.50 ± 2.1	20	35	36.13 ± 1.9
9	23	72.54 ± 3.5	21	38	33.67 ± 1.8
10	24	91.48 ± 4.6	22	39	39.82 ± 2.0
11	25	79.28 ± 3.9	23	40	66.01 ± 3.4
12	26	31.49 ± 1.8	24	41	76.15 ± 3.7

The pharmacokinetic properties of the most active compounds **27** and **35** were calculated theoretically by the use of online application PreADME https://preadmet.bmdrc.kr. As shown in table **5**, the tested compounds showed excellent human intestinal absorption that can reach 100 %. Furthermore, they have good plasma protein binding activity that exceeds 89.50 %, this data requires attention during the use of other drugs as it can displace them leading to increase the potency of the concurrent administered agents. It was also observed that the tested compounds have potency to penetrate the BBB more than doxorubicin. The results revealed that the tested compounds have better oral absorptivity and intestinal bioavailability than the standard drug used.

Oral bioavailability is considered an important factor for bioactive molecules development as therapeutic agents [27]. Lipinski used some molecular descriptors in formulating “Rule of Five” to assess the potential oral bioavailability and drug-likeness criteria of the molecules. Number of hydrogen bond donor, hydrogen bond acceptor sites, molecular weight, logP values [28, 29]. In addition, the total polar surface area (TPSA) and number of rotatable bonds (NROTB) represents additional key properties for drug bioavailability. The obtained results for compounds **27** and **35** revealed that compound **27** has no violations whereas compound **35** has one violation concerning Lipinski's rule. Number of rotatable bonds of compounds was <10, giving a hint about acceptable molecular flexibility with expected improved permeability and oral bioavailability. TPSA value was

<140 $Å^2$ which indicates a good predicted oral bioavailability. From these data, it could be suggested that both compounds can be used as good orally absorbed anticancer agent. All descriptors were calculated by the DruLito software and the results are listed in Table 6 [30].

Table 5: ADMET profile for compounds **27** and **35**

Comp.	HIA	PPB	BBB	MDCK	CaCo-2	CYP Inhibition	AMES Toxicity	Carcinogenecity	
								Mouse	Rat
27	97.77	94.00	1.13	0.0776	56.5653	Inhibitor	Mutagen	Positive	Negative
35	98.18	89.53	0.89	0.0459	29.3344	Inhibitor	Mutagen	Negative	Positive
Dox.	37.28	40.53	0.03	4.264	17.7512	Inhibitor	Non-Mutagen	Negative	Negative

HIA : Human Intestinal Absorption, **PPB**: Plasma Protein Binding, **BBB**: Blood Brain Barrier, **MDCK**: Maden Darby Canine Kidney, **CaCo-2:** Predict intestinal permeability and oral absorption of compounds

Table 6. Calculated Lipinski's rule of five and other *in silico* parameters for compounds **27** and **35**

[a]Molecular weight. [b]Number of hydrogen bond acceptors. [c]Number of hydrogen bond donors. [d]logarithm of compound partition coefficient between n-octanol and water. [e]Number of violations of Lipinski rule. [f]No. of rotatable bonds. [g]Topological polar surface area ($Å^2$).

In the present work, molecular modeling studies have been added as an important tool to understand the DNA binding data obtained experimentally for the most active compounds **27** and **35** (concerning DNA binding and in vitro antitumor screening, respectively) and rationalize their anticancer activity. Conformational analysis of the target compounds **27** and **35** has been obtained by use of the MM force-field as implemented in MOE, 2014. [31]. The lowest energy conformer of the selected active compounds was represented in Figure 3. X-ray crystallographic structure of the DNA dodecamer d(CGCAAATTTGCG) with its complex distamycin A was extracted from the Protein Data Bank (PDB code: 2DND, Deposited: 1988-08-29 Released: 1989-01-09) for the docking study [32]. Distamycin reference had produced with DNA bases Adenine, Cytosine and through hydrogen bonding network interactions with a calculated binding energy of -10.5674 kcal/A˚ mol (Figure 4a,b,

Comp.	Mwt[a]	HBA[b]	HBD[c]	LogP[d]	Lip. V[e]	NROTB[f]	TPSA[g]
27	448.15	6	0	3.786	0	8	85.66
35	510.18	8	0	4.014	1	9	96.28

2D and 3D view). Compounds **27** and **35** were docked into DNA minor groove, they showed hydrogen bonding interactions with binding energy of -10.2698 and -10.4532 kcal/A˚ mol, respectively (Figures 5a, b and 6a, b). Surface mapping study was performed for further investigation of activity pattern for compounds **27** and **35**. It showed a preference for more hydrophobic and H-bonding regions as more green and pink areas on the surface maps can be found. This provides additional explanation for their DNA binding activity. (Figure 7).

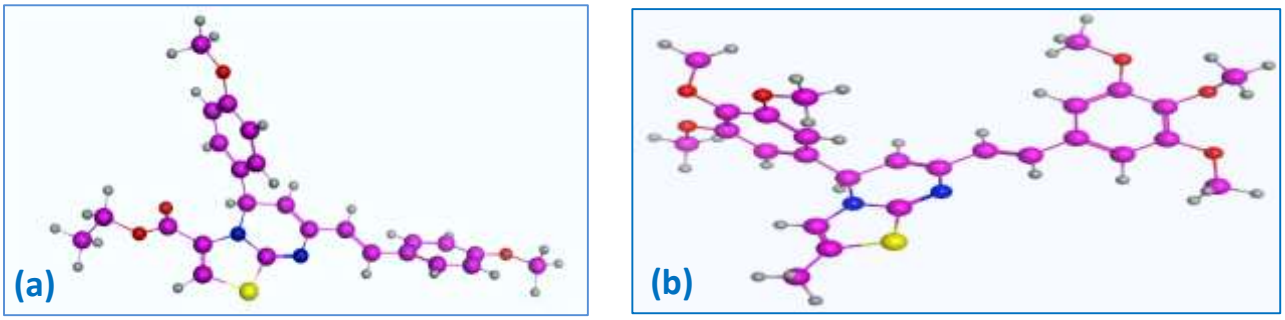


Figure 3: Lowest energy conformers of (a) compound 27 and (b) compound 35 with balls and cylinders

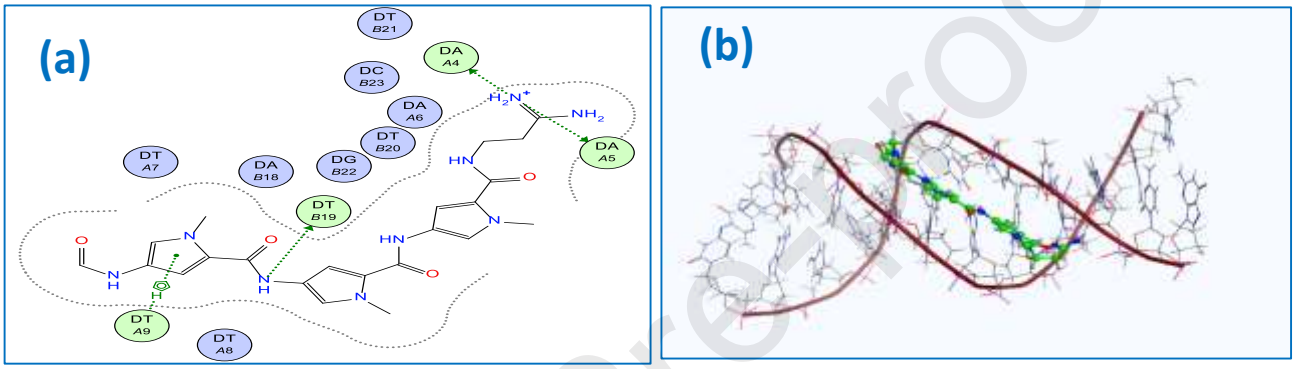


Figure 4: (a) 2D and (b) 3D binding mode and residues involved in the recognition of reference ligand Distamicin into DNA

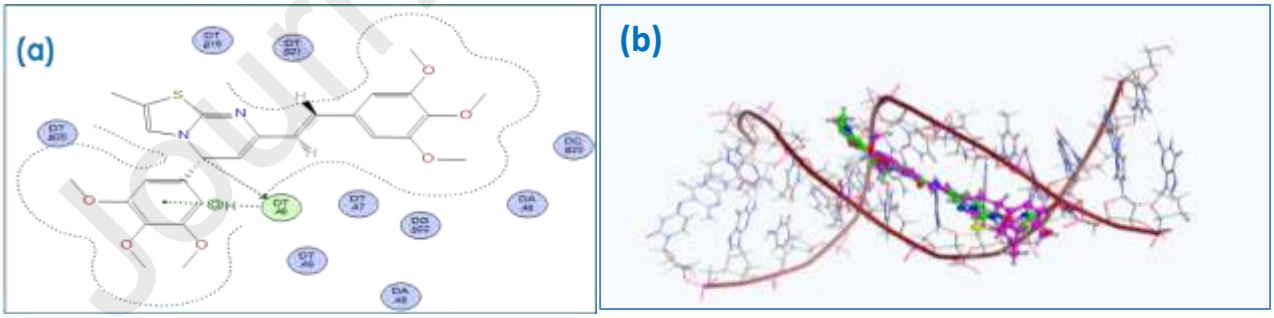


Figure 5: (a) 2D binding mode and residues involved in the recognition of compound 35 into DNA. (b) Overlay of compound 35 (pink) and Distamycin (green) into DNA minor groove.

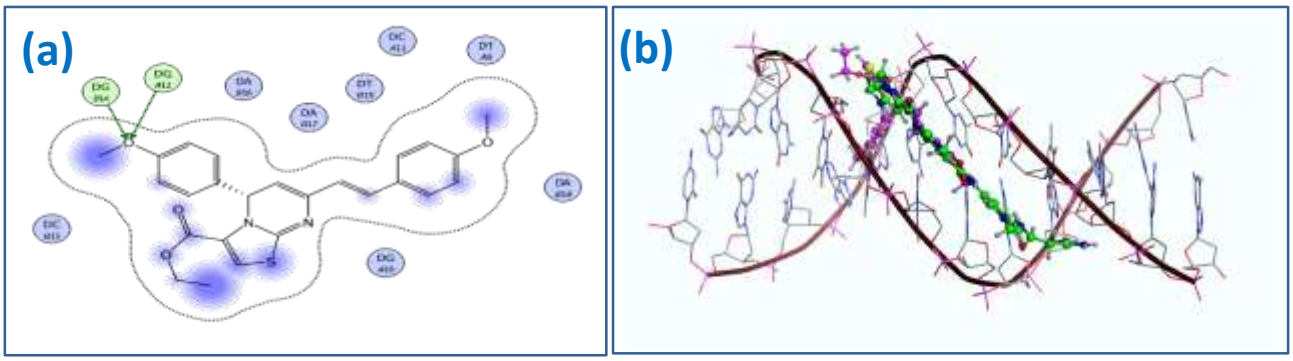


Figure 6: (a) 2D binding mode and residues involved in the recognition of compound 27 into DNA. (b) Overlay of compound 27 (pink) and Distamycin (green) into DNA minor groove.

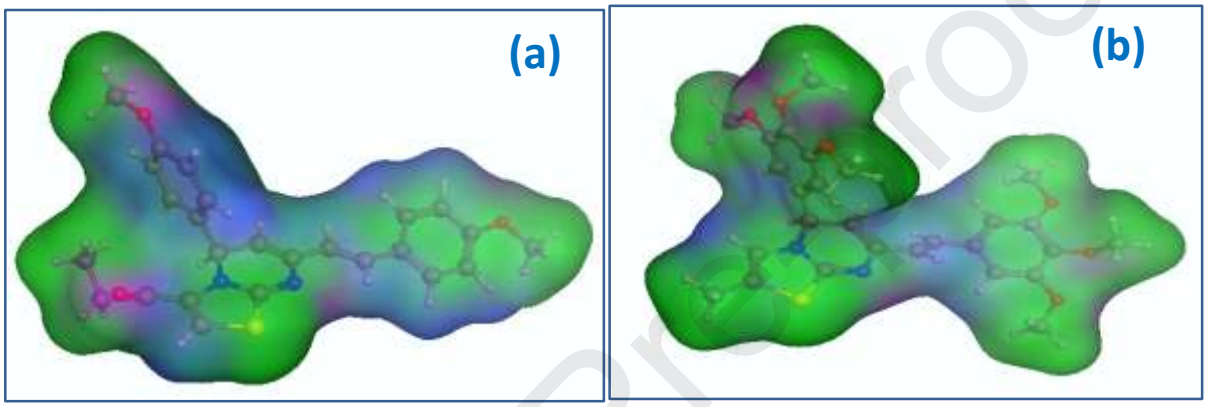


Figure 7: Surface maps for (a) compound 27 and (a) compound 35 (hydrophobic: green, H-bonding: pink, mild polar: blue)

A new series of thiazolopyrimidine derivatives were synthesized, their structures were confirmed by different spectroscopic techniques. Antitumor screening data revealed that compound **35** exhibited the highest activity among the entire synthesized products, it gives GI_{50}, TGI and LC_{50} values of 1.07, 6.61, 34.7 μM respectively. Also, it was proved to be an active compound as DNA binding agent with binding affinity **36.13 ± 1.9** with value that is comparable with that produced by doxorubicin which was used as positive standard. Furthermore, compound **27** was proved to be the most active DNA binding agent with binding affinity **28.38 ± 1.1**. The ADMET study of the synthesized compounds was also evaluated. Modeling study provides type of binding mode of compounds **27** and **35** into DNA minor groove via hydrogen bonding. The anticancer mechanism of action of these compounds could be attributed to DNA intercalation.

This manuscript submitted has not been previously published, nor is it before another journal for consideration"

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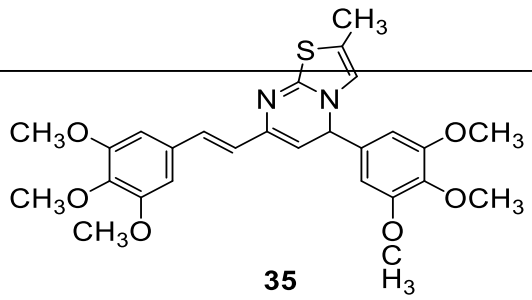
Highlights

- New series of thiazolopyrimidine derivatives has been synthesized.
- Antitumor screening according to NCI protocol against 60 different cell lines has been performed
- DNA binding affinity of the synthesized compounds has been calculated.
- Predicted ADMET data for the target compounds were also estimated.
- Molecular modeling study has been conducted.

Graphical Abstract

New Thiazolopyrimidine as anticancer agents: Synthesis, biological evaluation, DNA binding and ADMET study.

Sara T. Al-Rashood, Shymaa S. Elshahawy, Asmaa M. El-Qaias, Dina S. El-Behedy , Alshaimaa A. Hassanin, Selwan M. El-Sayed, Shahenda M. El-Messery*, Moataz A. Shaldam, Ghada S. Hassan



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