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Original article

Synthesis of 2-substituted-*N*-[4-(1-methyl-4,5-diphenyl-1*H*-imidazole-2-yl) phenyl]acetamide derivatives and evaluation of their anticancer activity

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ABSTRACT

In the present study 18 novel imidazole-(benz)azole and imidazole–piperazine derivatives were synthesized in order to investigate their probable anticancer activity. The structures of the compounds were confirmed by IR, ^{1}H NMR and EI-MS spectral data. Cytotoxicity (MTT), analysis of DNA synthesis and detection of apoptotic DNA assays were applied to determine anticancer activity of the compounds against colon (HT-29) and breast (MCF-7) carcinoma cell lines. Most of the compounds, showed greater activity against HT-29 cells than MCF-7 cells. Some of them indicated considerable cytotoxicity against both of the carcinogenic cell lines. However, their inhibitory activity on DNA synthesis was relatively poor. Anticancer activity screening results revealed that **11**, **12** and **13** were the most active compounds in the series. They exhibited significant cytotoxicity against both of the carcinogenic cell lines and caused DNA fragmentation of the HT-29 cells.

1. Introduction

DNA intercalators are the chemotherapeutics which exhibit anticancer activity by inserting between the base pairs of the double helix and causing a significant change of DNA conformation [1]. They bind to DNA by non-covalent interactions and constitute DNA–intercalator complex. The only recognized forces that maintain the stability of the DNA–intercalator complex, even more than DNA alone, are van der Waals, hydrogen bonding, polarization and hydrophobic forces [2–6]. The compounds which bear heteroatoms such as nitrogen, sulphur and oxygen increase the strength of the complex by forming hydrogen bonds with DNA. The force of interaction between compound and DNA usually correlates with the anticancer activity [7–9]. Besides, when one or more nitrogen heteroatoms exist on the chemical structure, intercalating chromophore possesses a polarized character and optimal interaction occurs [6]. Based on such reasons the presence of heteroatoms in the compounds plays an important role on exhibiting the anticancer activity.

Imidazole is a nitrogen containing heterocyclic ring which possesses biological and pharmaceutical importance. Thus, imidazole compounds have been an interesting source for researchers for more than a century [10]. Lepidiline A and B are the imidazole compounds which exhibit cytotoxicity against various types of human cancer cell lines at micromolar concentrations [11]. Dacarbazine [12], zoledronic acid [13], tipifarnib [14,15] and azathioprine [16] are the imidazole ring bearing anticancer agents. In addition to imidazole, some other azole and benzazole ring systems such as benzoxazole, benzothiazole, triazole, tetrazole, thiadiazole and thiazoline can be shown as pharmacophore groups which are responsible for the anticancer activity [17–22]. Piperazine is another nitrogenous moiety which often subjected to studies to develop new chemotherapeutic agents. Anticancer agents, razoxane (ICRF-154), dexrazoxsane (ICRF-187) and MST-16, are the compounds including this moiety [23,24].

Resistance improvement against the former anticancer agents creates a research area in development of new anticancer agents. Nevertheless, it is rather difficult to develop a new agent which can selectively inhibit the proliferation of abnormal cells with least or no effect on normal cells [25]. Therefore, cancer chemotherapy is very important for medicinal chemists and the studies are still being carried on to develop new chemotherapeutic agents that are probable to indicate activity on various cancer types [26,27].

Prompted from the observations described above and the chemotherapeutic value of the nitrogenous chemical scaffolds, synthesis and anticancer activity evaluation of some novel imidazole-(benz)azole and imidazole–piperazine derivatives were examined in this study.

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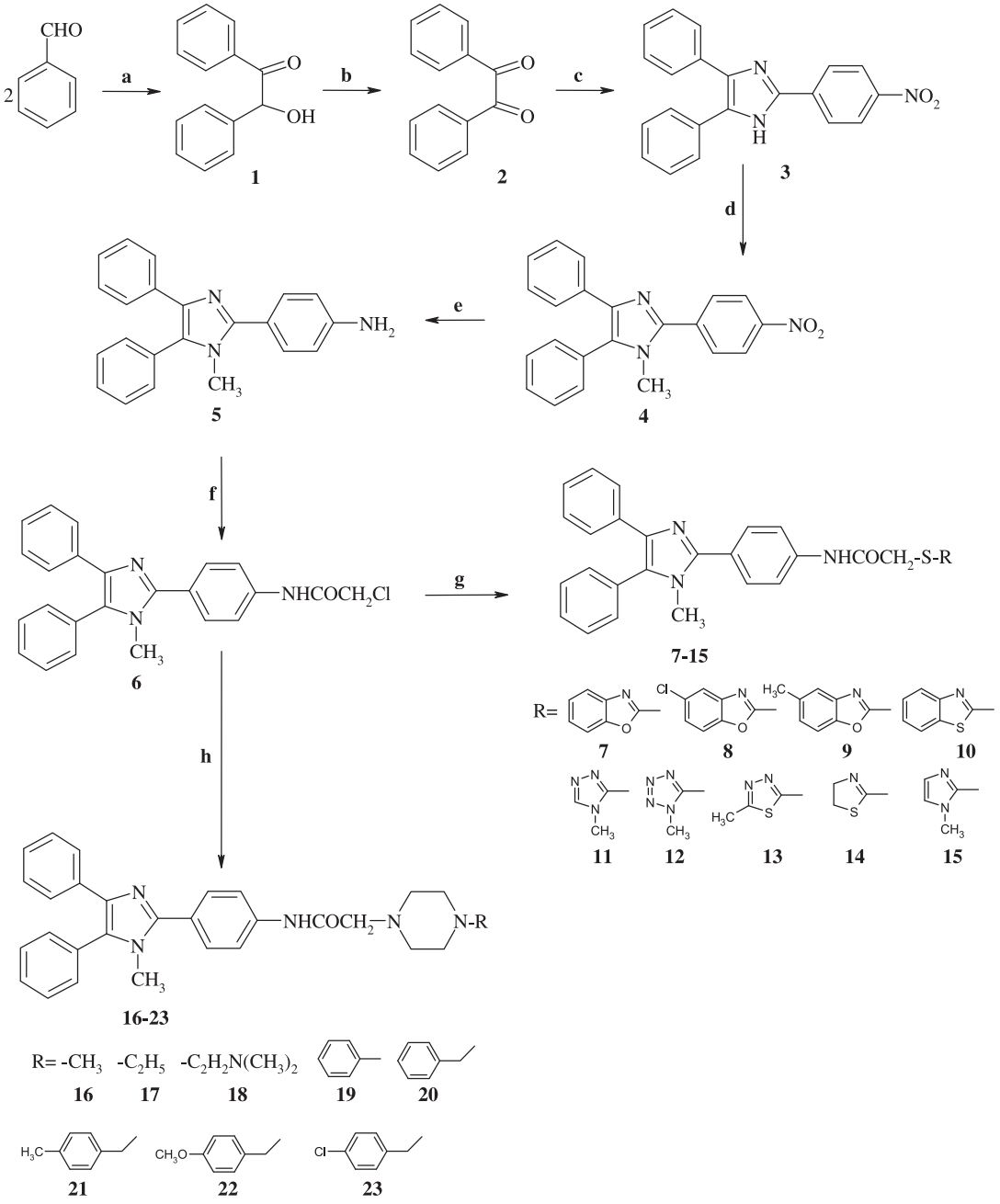
2. Results and discussion

2.1. Chemistry

For the synthesis of the target compounds the reaction sequence outlined in the Scheme 1 was followed. The reaction steps including dimerisation of benzaldehyde to benzoin (**1**), oxidation of the **1** to benzil (**2**), cyclisation of the **2** with 4-nitrobenzaldehyde to 2-(4-nitrophenyl)-4,5-diphenyl-1*H*-imidazole (**3**) and N-methylation of the **3** to 1-methyl-2-(4-nitrophenyl)-4,5-diphenyl-1*H*-imidazole (**4**), were performed according to previous studies [28–31]. Reduction of the **4** with Zn/HCl produced 1-methyl-2-(4-aminophenyl)-4,5-diphenyl-1*H*-imidazole (**5**). 2-Chloro-*N*-[4-(1-methyl-4,5-diphenyl-1*H*-imidazole-2-yl)phenyl]acetamide (**6**) was prepared via acetylation of the **5** with chloroacetyl chloride. At final step the **6** was reacted with appropriate thiol-(benz)azoles or corresponding piperazine derivatives to give 2-substituted-*N*-[4-(1-methyl-4,5-diphenyl-1*H*-imidazole-2-yl)phenyl]acetamide derivatives (**7**–**23**).

Structures of the obtained compounds were elucidated by spectral data. In the IR spectra, significant stretching bands belonging to N–H, C=O, C=N, C=C and deformation bands belonging to monosubstituted and 4-substitutedphenyl were observed at expected regions. The entire aromatic and aliphatic



Scheme 1. Synthesis of 2-substitued-*N*-[4-(1-methyl-4,5-diphenyl-1*H*-imidazole-2-yl) phenyl]acetamide derivatives (**6**–**23**) Reagents and conditions; **a**: NaCN, H_2O/EtOH, reflux 1 h, **b**: $(CH_3COO)_2Cu$, NH_4NO_3, AcOH, reflux 2 h, **c**: 4-Nitrobenzaldehyde, CH_3COONH_4, AcOH, reflux 3 h **d**: 1) NaH/THF, R.T. 15 min. 2) CH_3I reflux 3 h, **e**: Zn, EtOH/HCl, R.T. and then reflux 1 h, **f**: TEA, $ClCH_2COCl$, benzene, ice bath and then R.T. 1 h, **g**: Appropriate thiol-(benz)azole, K_2CO_3, acetone, reflux 12 h, **h**: Corresponding 1-substituted piperazine, K_2CO_3, acetone, reflux 24 h.

proton peaks in the 500 MHz ^{1}H NMR spectra were also recorded at estimated areas. The mass spectra (EI-MS) of the compounds showed [M + 1] peaks, in agreement with their molecular weight.

2.2. Biochemistry

Anticancer activity screening of the **6**–**23** against HT-29 and MCF-7 cell lines was performed at three steps. In the first step, cytotoxicities of the compounds were determined by MTT method. Afterwards, DNA synthesis inhibition was analyzed for the compounds possessing significant cytotoxic activity. Finally, apoptotic DNA fragmentation method was used for determination of internucleosomal DNA ladders, which are potent inducers of apoptosis, caused by compounds.

2.2.1. Cytotoxicity assay (MTT)

In the research for new anticancer agents, the most common screening methods employ cytotoxicity tests against a panel of cancer cell lines. These are high throughput screening assays, revealing compounds with the highest cytotoxic activity [32]. MTT, based on the ability of metabolically active cells to convert the pale yellow MTT dye to a spectrophotometrically quantifiable blue formazan product, is one of the most preferred cytotoxicity tests [33].

In the MTT test, HT-29 and MCF-7 cell lines were incubated with the various concentrations (0.64, 1.6, 6.4, 16, 32 and 80 μg/mL) of **6**–**23**. After the completion of incubation period (24 h), cytotoxic activity of the compounds was examined and IC_{50} values were calculated. Anticancer agent cisplatin was used as a positive control.

Compounds subjected to cytotoxicity test were designed to contain two different pharmacophore groups which exist on the chemical structures of some anticancer agents and are estimated to be responsible for the anticancer activity. N-[4-(4,5-diphenyl-1*H*-imidazole-2-yl)phenyl]acetamide substructure was fixed in all of the compounds. This substructure was substituted from second position of the acetamide moiety in order to classify the compounds in two different groups. The first group was constituted by using different azole and benzazole heterocycles and named imidazole-(benz)azoles. The second group was imidazole–piperazines and formed by substitution of corresponding piperazine derivatives. The starting compound (**6**) was also implicated to cytotoxicity test in order to determine the contribution of variable groups to activity.

Cytotoxic activity of the compounds against HT-29 and MCF-7 cell lines is presented in Tables 1 and 2. In the first group the **7**, **10**, **11**, **12** and **13** displayed higher cytotoxic activity against both of the cell lines than the **6**. Cytotoxicity of the **15** was lower than that of the **6.** The other thiol-(benz)azole moiety bearing compounds **8**, **9** and **14** showed approximate cytotoxic activity to the **6**. These results suggest that benzoxazole-2-thiol, benzothiazole-2-thiol, 1,2,4-triazole-5-thiol, 1,2,3,4-tetrazole-5-thiol and 1,2,4-thiadiazole-3-thiol moieties supply a substantial contribution to increase of the cytotoxicity. On the other hand, determination of lower cytotoxicity with the **15** than the **6** indicates that substitution of the **6** with 1-methyl-imidazole-2-thiol causes an activity decrease. The observed findings also refer that 5-methyl-benzoxazole-2-thiol, 5-chloro-benzoxazole-2-thiol and thiazoline-2-thiol substituted compounds have no effect on the cytotoxicity.

In the second group, most of the compounds exhibited lower cytotoxicity against both of the cell lines than the **6** (Tables 1 and 2). In the 0.64–32 μg/mL concentrations range, only the **19** and **23** revealed greater cytotoxic activity than the **6**. However, when concentrations were raised to 80 μg/mL, cytotoxic activity of the **6** increased significantly and seemed as higher than those of the **19** and **23**. Cytotoxicity test results of the **16**–**23** demonstrate that substitution of the **6** with 1-substituted piperazine derivatives creates no important effect on the activity.

When cytotoxic activity of the imidazole-(benz)azoles is compared with that of the imidazole–piperazines, it can be clearly seen that the first group compounds display greater cytotoxicity. The **7**, **11**, **12** and **13** are the most cytotoxic compounds in the series. Moreover, cytotoxicities of the **11**, **12** and **13** against HT-29 cells and cytotoxicities of the **11** and **13** against MCF-7 cells are very close to that of cisplatin. These results show that thiol-azole and thiol-benzazole moieties have more contribution to increase cytotoxic activity than piperazine ring. Such positive contribution to cytotoxicity can be explained by hydrogen bonding capacity of the first group. The **7**–**15** bear a sulphur atom between N-[4-(4,5-diphenyl-1*H*-imidazole-2-yl)phenyl]acetamide substructure and azole or benzazole rings. On the other hand, the second group compounds **16**–**23** are constituted by a direct linkage between same substructure and piperazine ring. Hence, the imidazole-(benz)

Table 1
Cytotoxic activity of the compounds **6**–**23** against HT-29 cell line.

Compounds	Concentration (μg/mL)						IC_{50} (μg/mL)
	0.64	1.6	6.4	16	32	80	
6	7.2 ± 1.8	14.4 ± 2.2	15.1 ± 3.0	23.7 ± 1.6	53.2 ± 3.3*	76.1 ± 3.4*	28.7
7	24.8 ± 2.1	41.0 ± 2.3	44.9 ± 2.5	56.2 ± 2.7*	79.3 ± 1.7*	81.5 ± 1.5*	11
8	11.9 ± 2.2	17.4 ± 4.3	29.4 ± 1.5	42.5 ± 1.0	61.9 ± 2.7*	79.6 ± 2.0*	20.6
9	11.0 ± 1.4	15.5 ± 2.1	25.6 ± 2.7	37.7 ± 2.3	59.5 ± 2.2*	69.8 ± 2.1*	22.4
10	9.8 ± 1.7	15.3 ± 3.3	36.8 ± 3.2	47.2 ± 1.8	66.0 ± 2.3*	82.7 ± 2.5*	18
11	32.0 ± 1.7	40.8 ± 2.9	43.1 ± 3.4	59.1 ± 1.2*	86.1 ± 2.7*	88.0 ± 2.5*	10.7
12	38.0 ± 1.3	49.8 ± 2.3	60.6 ± 2.8*	72.5 ± 2.0*	84.0 ± 3.6*	89.0 ± 2.8*	1.6
13	34.8 ± 2.1	39.9 ± 2.5	73.0 ± 3.2*	83.3 ± 1.4*	87.1 ± 2.5*	90.5 ± 3.2*	2.7
14	16.8 ± 2.0	30.4 ± 2.7	31.0 ± 1.3	43.3 ± 2.7	59.4 ± 1.5*	75.2 ± 2.7*	21
15	13.6 ± 1.9	23.8 ± 2.6	30.7 ± 2.9	36.5 ± 0.4	41.1 ± 1.6	69.4 ± 2.3*	47.5
16	18.8 ± 2.2	27.4 ± 1.2	28.8 ± 2.6	33.7 ± 5.3	49.1 ± 3.0	53.1 ± 2.4	35.5
17	7.4 ± 1.3	13.9 ± 2.0	17.2 ± 2.8	28.4 ± 2.1	38.8 ± 1.8	51.2 ± 2.4*	75
18	3.1 ± 2.7	8.0 ± 1.8	12.2 ± 2.9	28.2 ± 2.8	29.8 ± 2.9	45.4 ± 3.0	>80
19	17.3 ± 1.9	26.0 ± 0.5	46.5 ± 2.2	55.4 ± 3.3*	60.5 ± 2.4*	63.7 ± 3.0*	8.6
20	15.5 ± 0.8	26.0 ± 2.6	26.8 ± 5.4	37.0 ± 3.2	40.0 ± 3.1	54.5 ± 1.2*	65.5
21	15.4 ± 2.6	26.4 ± 1.3	27.7 ± 2.7	30.6 ± 2.1	42.3 ± 1.1	54.2 ± 3.1*	62
22	5.2 ± 1.6	10.1 ± 2.5	17.8 ± 3.2	21.8 ± 1.3	29.9 ± 2.5	51.2 ± 2.6*	77.3
23	11.3 ± 2.2	16.4 ± 2.8	41.5 ± 4.6	52.9 ± 3.2*	55.6 ± 1.7*	60.7 ± 1.6*	12
Cisplatin	41.3 ± 1.4	48.1 ± 2.1	71.3 ± 1.7*	89.1 ± 3.6*	98.2 ± 2.4*	99.3 ± 1.3*	1.7

Results are expressed as the mean % of MTT absorbance (ratio of absorbance in test compound treated and control cells). Data points represent means of three independent experiments ±SD of twelve independent wells. $p < 0.05$. *Significantly different from control cells.

Table 2
Cytotoxic activity of the compounds **6**–**23** against MCF-7 cell line.

Compounds	Concentration (μg/mL)						IC_{50} (μg/mL)
	0.64	1.6	6.4	16	32	80	
6	3.4 ± 0.7	4.3 ± 1.4.	14.2 ± 1.0	15.2 ± 2.2	42.8 ± 2.0	70.0 ± 0.9*	42.6
7	18.7 ± 1.4	27.4 ± 3.9	42.3 ± 1.0	56.0 ± 1.9*	71.5 ± 1.3*	76.3 ± 2.4*	11.2
8	2.4 ± 0.9	2.5 ± 2.1	27.7 ± 3.6	40.0 ± 1.8	59.7 ± 1.2*	69.1 ± 2.2*	21.7
9	1.1 ± 1.1	1.9 ± 0.1	24.6 ± 0.5	40.3 ± 1.5	57.7 ± 0.4*	65.1 ± 2.1*	22.4
10	0.9 ± 0.6	2.1 ± 1.3	27.0 ± 3.4	50.3 ± 1.8*	59.0 ± 1.2*	76.6 ± 2.2*	15.8
11	33.1 ± 2.2	42.8 ± 1.6	59.2 ± 2.6*	61.2 ± 1.0*	89.2 ± 0.8*	89.3 ± 1.9*	3.2
12	21.9 ± 1.6	34.3 ± 1.1	55.9 ± 0.9*	63.7 ± 1.6*	77.5 ± 1.4*	77.7 ± 3.4*	4.5
13	35.1 ± 1.3	37.5 ± 0.7	67.8 ± 1.2*	74.9 ± 1.0*	83.4 ± 1.9*	85.2 ± 2.8*	3.2
14	10.3 ± 1.5	16.2 ± 1.0	18.6 ± 1.8	41.8 ± 1.2	52.5 ± 2.3*	70.0 ± 1.4*	26.7
15	10.3 ± 1.1	17.5 ± 0.8	27.2 ± 1.3	32.4 ± 2.7	43.4 ± 1.8	64.0 ± 3.3*	46.5
16	1.1 ± 0.9	2.9 ± 1.4	28.6 ± 1.6	30.1 ± 1.9	46.2 ± 3.4	50.3 ± 2.7*	76.5
17	4.7 ± 0.4	14.6 ± 0.9	16.4 ± 1.8	32.8 ± 3.2	38.6 ± 1.3	46.3 ± 2.3	>80
18	1.2 ± 1.1	1.9 ± 0.8	11.1 ± 1.2	19.4 ± 1.8	25.8 ± 2.4	34.7 ± 2.9	>80
19	12.1 ± 1.0	21.3 ± 2.5	37.7 ± 1.5	59.6 ± 0.8*	63.6 ± 1.8*	72.5 ± 3.7*	10.7
20	7.2 ± 0.9	13.3 ± 2.0	23.8 ± 1.3	28.3 ± 2.0	33.6 ± 1.9	49.5 ± 2.4	>80
21	9.4 ± 2.2	19.3 ± 1.6	21.8 ± 3.4	26.0 ± 2.3	31.5 ± 0.9	49.3 ± 2.8	>80
22	1.0 ± 0.9	27.3 ± 3.2	29.0 ± 1.6	12.0 ± 2.3	17.7 ± 2.4	34.5 ± 1.1	>80
23	4.8 ± 2.1	11.8 ± 1.9	33.4 ± 1.0	46.2 ± 2.5	52.8 ± 3.5*	52.9 ± 2.4*	21
Cisplatin	38.4 ± 0.7	44.7 ± 1.9	67.1 ± 3.1*	86.3 ± 2.7*	98.5 ± 2.2*	99.4 ± 1.6*	2.6

Results are expressed as the mean percent of MTT absorbance (ratio of absorbance in test compound treated and control cells). Data points represent means of three independent experiments ±SD of twelve independent wells. $p < 0.05$. *Significantly different from control cells.

azoles have more heteroatoms which may provide more capability to interact with DNA by hydrogen bonds. Due to the correlation between force of binding to DNA and anticancer activity, greater cytotoxic activity with the first group can be related to the number of heteroatoms.

2.2.2. Analysis of DNA synthesis

This immunostaining procedure is based on measuring the incorporation of bromodeoxyuridine (BrdU) into nuclear DNA in place of thymidine during the S-phase of the cell cycle using specific anti-BrdU antibodies [34]. Hence, such method provides a colorimetric measurement for DNA synthesis inhibition ratio of the carcinogenic cells.

DNA synthesis of the carcinogenic cell lines were analyzed for the **7**, **11**, **12** and **13** which indicated significant cytotoxic activity with MTT test. Cisplatin was used as a positive control. For 24 h and 48 h time periods, HT-29 and MCF-7 cells were incubated with three different concentrations of the compounds that were determined according to their IC_{50}. Tested compounds showed time- and dose-dependent inhibitory activity on DNA synthesis of both cell lines.

DNA synthesis inhibitory activity of the compounds on HT-29 cells is presented in Fig. 1. On the HT-29 cell line, applied concentrations of the **7** are approximate to those of the **11**. When compared the DNA synthesis inhibitory activity of these compounds each other, it is clear that the **11** has higher activity than the **7**. After comparing inhibitory activity of the **11** with that of the **12** at the concentrations of 3.2 μg/mL and 6.4 μg/mL, it is determined that the **12** is more potent to inhibit the DNA synthesis than the **11**. The **13** is the most effective compound against HT-29 cell line because at the concentration of 1.6 μg/mL, DNA synthesis inhibitory activity of the **13** is greater than that of the **12**. All of these comparisons enable to conclude the inhibitory activity ordering of the compounds as **13** > **12**>**11** > **7**.

DNA synthesis inhibitory activity of the compounds on MCF-7 cells is presented in Fig. 2. If the DNA synthesis inhibitory activity of the compounds on MCF-7 cell line is compared each other by following the same way used for HT-29 cells, it can be noticed that the activity orderings of the compounds on HT-29 and MCF-7 cells seem to be quite similar. However, such activity is more notable against HT-29 cell line. This finding suggests that HT-29 cells are more sensitive to the compounds than MCF-7 cells.

Consequently, analysis of DNA synthesis indicates that the **7**, **11**, **12** and **13** have greater inhibitory effects on DNA synthesis of the HT-29 cell line. However, DNA synthesis inhibitory activity of the cisplatin against both of the cell lines is significantly greater than that of the tested compounds. Furthermore, the results of DNA synthesis analysis are not as significant as cytotoxicity test results. These findings demonstrate that anticancer ability of the **7**, **11**, **12** and **13** is not related to their DNA synthesis inhibitory activity.

2.2.3. Detection of apoptotic DNA fragmentation

This method was carried out for the most cytotoxic compounds against HT-29 cell line hence this cell line was more sensitive to the compounds. HT-29 cells were incubated with the compounds at two different concentrations. IC_{50} and lower concentrations were preferred for this purpose. When incubation period (24 h) was over, DNA of the cells were isolated by using apoptotic DNA ladder kit and monitored in UV gel visualization system. Activity of the compounds on genomic DNA of the HT-29 cell line is given in Fig. 3.

As seen in Fig. 3, IC_{50} of the **11**, **12** and **13** cause DNA fragmentation (lanes 2, 3 and 4). However, when applied concentrations are decreased DNA ladders disappear (lanes 6, 7 and 8). The **7** makes no damage on DNA of the HT-29 cells at the applied concentrations. These findings can be explained by different constituents of the tested compounds. Benzoxazole ring is a fragment of the **7** and includes two heteroatoms. On the other hand, the **11**, **12** and **13** involve 1,2,4-triazole, 1,2,3,4-tetrazole and 1,2,4-thiadiazole rings which bear more than two heteroatoms. Therefore, polarization characters and hydrogen bonding capacities of the **11**, **12** and **13** are expected to be better than those of the **7**. As a result, stronger interaction between DNA and **11**, **12** and **13**, which probably cause to DNA fragmentation, may occur.

3. Conclusion

The preliminary *in vitro* anticancer investigation of novel imidazole-(benz)azole and imidazole–piperazine derivatives has indicated the anticancer potency of the tested compounds. In general, imidazole-(benz)azole compounds exhibited greater anticancer activity than imidazole–piperazine derivatives. Especially the compounds **11**, **12** and **13** indicated significant anticancer activity against colon carcinoma cell line. These three compounds showed

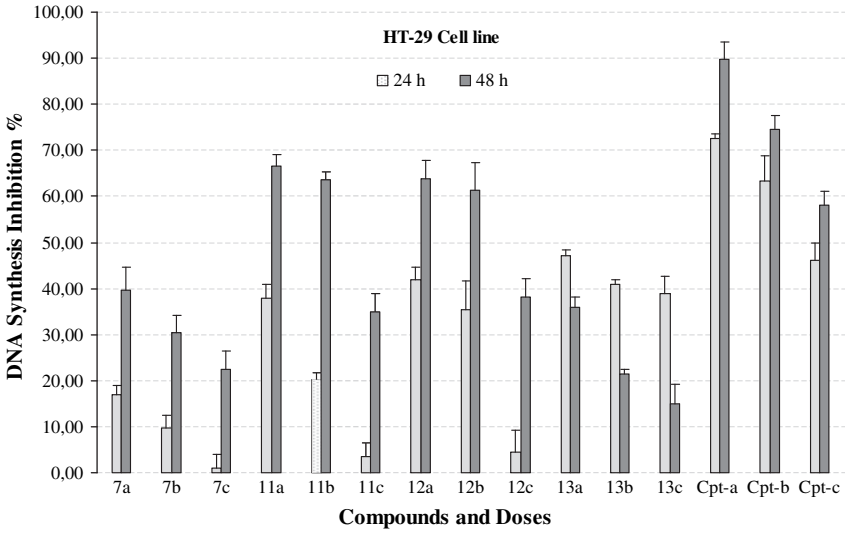


Fig. 1. DNA synthesis inhibitory activity of the compounds **7**, **11**, **12** and **13** on HT-29 cells. Mean percent absorbance of the untreated (assessed in the presence of DMSO used as a solvent and assumed as 0%), and three different concentrations (**7a** 11 μg/mL; **7b** 6.4 μg/mL; **7c** 3.2 μg/mL; **11a** 10 μg/mL; **11b** 6.4 μg/mL; **11c** 3.2 μg/mL; **12a** 4 μg/mL; **12b** 3.2 μg/mL; **12c** 1.6 μg/mL; **13a** 2.7 μg/mL; **13b** 1.6 μg/mL; **13c** 0.64 μg/mL; **Cpt-a** 3.2 μg/mL; **Cpt-b** 1.7 μg/mL; **Cpt-c** 0.64 μg/mL) of test compounds and cisplatin were given. Data points represent means for three independent experiments ±SD of nine independent wells. $p < 0.05$.

substantial cytotoxicity and caused DNA fragmentation of the HT-29 cells. However, they displayed poor inhibitory activity on DNA synthesis. Such results refer that anticancer ability of the compounds arises after DNA synthesis of the cells has been completed. The results also reveal that anticancer ability of the compounds is related to their cytotoxic and apoptotic effects on HT-29 cell lines.

In the present study, 1,2,4-triazole, 1,2,3,4-tetrazole and 1,2,4-thiadiazole rings are the main heterocycles that are responsible for the anticancer activity. Significant contribution to anticancer activity with the presence of these ring systems have encouraged us to make some modifications on basic structure of obtained compounds to achieve more active derivatives in further studies. For this purpose it is considered to synthesize some new compounds that carry 1,2,4-triazole-5-thiol, 1,2,3,4-tetrazole-5-thiol and 1,2,4-thiadiazole-3-thiol moieties and show structural similarity to the compounds presented in this study. First choice to implement this synthesis strategy can be replacement of an alternative heterocyclic ring system instead of 4,5-diphenylimidazole, which is participated in all of the tested compounds.

4. Materials and methods

4.1. Chemistry

Chemicals used in syntheses were obtained from Acros, Aldrich, Alfa-Aesar, Fluka or Merck chemical companies. Melting points (m.p.) of the target compounds were measured in Electrothermal 9001 Digital Melting Point Apparatus and uncorrected. IR and ^{1}H NMR spectra were recorded on Shimadzu, 8400 FTIR spectrometer as KBr pellets and on Bruker UltraShield 500 MHz spectrometer in DMSO-d_6, respectively. EI-MS data were obtained on Quattromicro Mass spectrometer.

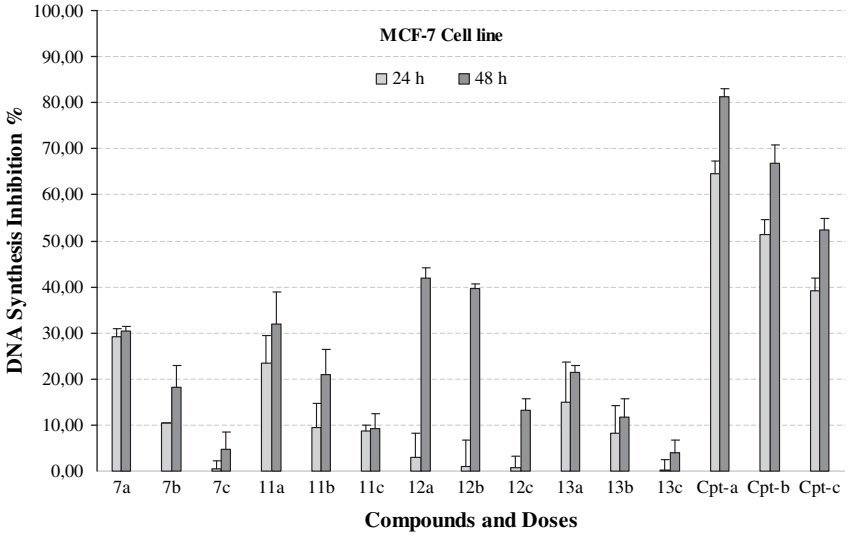


Fig. 2. DNA synthesis inhibitory activity of the compounds **7**, **11**, **12** and **13** on MCF-7 cells. Mean percent absorbance of the untreated (assessed in the presence of DMSO used as a solvent and assumed as 0%), and three different concentrations (**7a** 11.2 μg/mL; **7b** 6.4 μg/mL; **7c** 3.2 μg/mL; **11a** 4.2 μg/mL; **11b** 3.2 μg/mL; **11c** 1.6 μg/mL; **12a** 5 μg/mL; **12b** 3.2 μg/mL; **12c** 1.6 μg/mL; **13a** 2.8 μg/mL; **13b** 1.6 μg/mL; **13c** 0.64 μg/mL; **Cpt-a** 3.2 μg/mL; **Cpt-b** 1.7 μg/mL; **Cpt-c** 0.64 μg/mL) of test compounds and cisplatin were given. Data points represent means for three independent experiments ±SD of nine independent wells. $p < 0.05$.

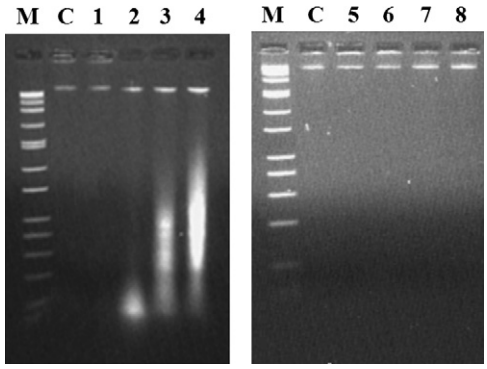


Fig. 3. Effects of the compounds **7**, **11**, **12** and **13** on genomic DNA of HT-29 cells. M: DNA marker (10,000-50 bp); C: Control (DNA of HT-29 cell line); 1: DNA of HT-29 cell line treated with 11 mg/mL of **7**; 2: DNA of HT-29 cell line treated with 10 mg/mL of **11**; 3: DNA of HT-29 cell line treated with 1.6 mg/mL of **12**; 4: DNA of HT-29 cell line treated with 2.7 mg/mL of **13**; 5: DNA of HT-29 cell line treated with 6.4 mg/mL of **7**; 6: DNA of HT-29 cell line treated with 6.4 mg/mL of **11**; 7: DNA of HT-29 cell line treated with 1 mg/mL of **12**; 8: DNA of HT-29 cell line treated with 1.6 mg/mL of **13**.

*4.1.1. Synthesis of the starting materials (**1**–**4**)*

The starting materials; benzoin (**1**) (m.p. 135 °C; lit. m.p. 134 [28]), benzil (**2**) (m.p. 94 °C; lit. m.p. 94–95 °C [29]), 2-(4-nitrophenyl)-4,5-diphenyl-1*H*-imidazole (**3**) (m.p. 240 °C; lit. m.p. 242 °C [30]) and 1-methyl-2-(4-nitrophenyl)-4,5-diphenyl-1*H*-imidazole (**4**) (m.p. lit. 196 °C; m.p. 198 °C [31]), were synthesized according to previous studies.

*4.1.2. 1-Methyl-2-(4-aminophenyl)-4,5-diphenyl-1H-imidazole (**5**)*

The compound **4** (26.625 g, 75 mmol) was dissolved in the mixture of 200 mL EtOH and 200 mL HCl (25%). Zinc dust (7.3125 g, 1125 mmol) was divided into ten portions (0.73125 g × 10) and each portion was added to the stirring solution in 15 min intervals. Once the addition of the zinc was completed, reaction mixture was refluxed for 1 h. Hot solution was allowed to cool down, poured into ice water and then neutralized by using 10% NaOH solution. The precipitate was extracted with chloroform (3 × 100 mL). The extracts were combined and filtered over anhydrous Na_2SO_4. Solvent was evaporated and the white residue was recrystallized from ethanol to give the **5**. Yield 66%. m.p. 206 °C; lit. m.p. 204 °C [31].

*4.1.3. 2-Chloro-N-[4-(4,5-diphenyl-1H-imidazole-2-yl)phenyl] acetamide (**6**)*

The **5** (16.25 g, 50 mmol) was dissolved in 100 mL of benzene and 8.5 mL (60 mmol) of triethylamine was added. This mixture was allowed to stir on an ice bath. 4.8 mL (60 mmol) of chloroacetyl chloride and 10 mL of benzene mixture was added drop by drop. After completion of dropping, reaction mixture was stirred for 1 h at room temperature (R.T.). Benzene was evaporated and brown residue was recrystallized from ethanol to give the **6**. Yield 78%. m.p. 200 °C. IR (KBr) ν_{maks} (cm^{-1}): 3370 (N–H), 1682 (C=O), 1605–1400 (C=C and C=N), 841 (1,4-disubstituted benzene), 772–696 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 3.48 (3H, s, imidazole N–CH_3), 4.31 (2H, s, CO–CH_2), 7.13–7.25 (3H, m, C_6H_5–H), 7.43–7.57 (7H, m, C_6H_5–H), 7.78 (4H, s, C_6H_4–H), 10.50 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 402.

*4.1.4. General procedure for 2-substituted-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl] acetamide derivatives (**7**–**15**)*

A mixture of the **6** (0.803 g, 2 mmol), appropriate (benz)azole-thiol derivative (2 mmol) and K_2CO_3 (0.276 g, 2 mmol) in acetone (30 mL) was refluxed for 12 h. After cooling, the solution was evaporated until dryness. The residue was washed with water and recrystallized from ethanol to give the **7**–**15**.

*4.1.4.1. 2-Benzoxazole-2-yl-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**7**).* Yield 78%. m.p. 232 °C. IR (KBr) ν_{maks}(cm^{-1}): 3349 (N–H), 1682 (C=O), 1599–1395 (C=C and C=N), 837 (1,4-disubstituted benzene), 746–702 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 3.48 (3H, s, imidazole N–CH_3), 4.46 (2H, s, CO–CH_2), 7.13–7.24 (3H, m, C_6H_5–H), 7.33–7.38 (2H, m, benzoxazole $C_{3,4}$–H), 7.42–7.56 (7H, m, C_6H_5–H), 7.64–7.69 (2H, m, benzoxazole $C_{2,5}$–H), 7.78 (4H, s, C_6H_4–H), 10.75 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 517.

*4.1.4.2. 2-(5-Methyl-benzoxazole-2-yl)-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**8**).* Yield 84%. m.p. 215 °C. IR (KBr) ν_{maks}(cm^{-1}): 3350 (N–H), 1676 (C=O), 1599–1395 (C=C and C=N), 837 (1,4-disubstituted benzene), 775–698 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.40 (3H, s, benzoxazole –CH_3), 3.48 (3H, s, imidazole N–CH_3), 4.43 (2H, s, CO–CH_2), 7.12–7.25 (4H, m, C_6H_5–H and benzoxazole C_4–H), 7.42–7.56 (9H, m, C_6H_5–H and benzoxazole $C_{2,5}$–H), 7.77 (4H, s, C_6H_4–), 10.75 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 531.

*4.1.4.3. 2-(5-Chloro-benzoxazole-2-yl)-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**9**).* Yield 73%. m.p. 217 °C. IR (KBr) ν_{maks}(cm^{-1}): 3349 (N–H), 1676 (C=O), 1599–1397 (C=C and C=N), 829 (1,4-disubstituted benzene), 775–698 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 3.49 (3H, s, imidazole N–CH_3), 4.47 (2H, s, CO–CH_2), 7.12–7.25 (3H, m, C_6H_5–H), 7.38–7.58 (8H, m, C_6H_5–H and benzoxazole C_4–H), 7.72 (2H, m, benzoxazole $C_{2,5}$–H), 7.77 (4H, s, C_6H_4–H), 10.75 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 551.

*4.1.4.4. 2-Benzothiazole-2-yl-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**10**).* Yield 87%. m.p. 226 °C. IR (KBr) ν_{maks}(cm^{-1}): 3339 (N–H), 1676 (C=O), 1599–1395 (C=C and C=N), 839 (1,4-disubstituted benzene), 762–708 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 3.48 (3H, s, imidazole N–CH_3), 4.47 (2H, s, CO–CH_2), 7.12–7.24 (3H, m, C_6H_5–H), 7.38–7.58 (9H, m, C_6H_5–H and benzothiazole $C_{3,4}$–H), 7.77 (4H, s, C_6H_4–H), 7.86 (H, d, J = 8.10 Hz, benzothiazole C_5–H), 8.05 (H, d, J = 7.97 Hz, benzothiazole C_2–H), 10.74 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 533.

*4.1.4.5. 2-(4-Methyl-4H-1,2,4-triazole-3-yl)-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**11**).* Yield 81%. m.p. 259 °C. IR (KBr) ν_{maks}(cm^{-1}): 3237 (N–H), 1694 (C=O), 1605–1398 (C=C and C=N), 845 (1,4-disubstituted benzene), 775–696 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 3.48 (3H, s, imidazole N–CH_3), 3.63 (3H, s, triazole N–CH_3), 4.12 (2H, s, CO–CH_2), 7.12–7.24 (3H, m, C_6H_5–H), 7.42–7.57 (7H, m, C_6H_5–H), 7.75 (4H, q, $J_{a,a'}$ = 8.89 Hz, $J_{b,b'}$ = 8.92 Hz, C_6H_4–H), 8.60 (H, s, triazole C_2–H), 10.60 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 481.

*4.1.4.6. 2-(1-Methyl-1H-1, 2, 3, 4-tetrazole-5-yl)-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**12**).* Yield 85%. m.p. 251 °C. IR (KBr) ν_{maks}(cm^{-1}): 3268 (N–H), 1692 (C=O), 1607–1395 (C=C and C=N), 841 (1,4-disubstituted benzene), 783–702 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 3.48 (3H, s, imidazole N–CH_3), 4.02 (3H, s, tetrazole N–CH_3), 4.35 (2H, s, CO–CH_2), 7.12–7.24 (3H, m, C_6H_5–H),

7.42–7.56 (7H, m, C_6H_5–H), 7.76 (4H, q, $J_{aa'} = 8.82$ Hz, $J_{bb'} = 8.78$ Hz, C_6H_4–H), 10.60 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 482.

*4.1.4.7. 2-(5-Methyl-1,2,4-thiadiazole-3-yl)-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**13**).* Yield 82%. m.p. 234 °C. IR (KBr) ν_{maks}(cm^{-1}): 3349 (N–H), 1676 (C=O), 1599–1391 (C=C and C=N), 839 (1,4-disubstituted benzene), 775–698 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.69 (3H, s, thiadiazole $-CH_3$), 3.48 (3H, s, imidazole N–CH_3), 4.33 (2H, s, CO–CH_2), 7.12–7.24 (3H, m, C_6H_5–H), 7.42–7.56 (7H, m, C_6H_5–H), 7.76 (4H, s, C_6H_4–H), 10.59 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 498.

*4.1.4.8. 2-Thiazoline-2-yl-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**14**).* Yield 76%. m.p. 215 °C. IR (KBr) ν_{maks}(cm^{-1}): 3339 (N–H), 1676 (C=O), 1599–1395 (C=C and C=N), 839 (1,4-disubstituted benzene), 777–704 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 3.46–3.52 (5H, s, imidazole N–CH_3 and thiazoline C_3–2H), 4.12–4.18 (4H, m, CO–CH_2 and thiazoline C_2–2H), 7.12–7.24 (3H, m, C_6H_5–H), 7.42–7.56 (7H, m, C_6H_5–H), 7.76 (4H, s, C_6H_4–H), 10.59 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 485.

*4.1.4.9. 2-(1-Methyl-1H-imidazole-2-yl)-sulfanyl-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**15**).* Yield 79%. m.p. 220 °C. IR (KBr) ν_{maks}(cm^{-1}): 3241 (N–H), 1682 (C=O), 1613–1402 (C=C and C=N), 849 (1,4-disubstituted benzene), 775–696 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.95 (3H, s, imidazole N–CH_3), 3.48 (3H, s, 4,5-diphenyl-imidazole N–CH_3), 4.23 (2H, m, CO–CH_2), 6.96–7.14 (3H, m, C_6H_5–H), 7.38–7.54 (7H, m, C_6H_5–H), 7.75 (4H, q, $J_{aa'} = 7.64$ Hz, $J_{bb'} = 7.67$, C_6H_4–H), 7.97 (H, d, $J = 8.04$ Hz, imidazole C_3–H), 8.22 (H, d, $J = 7.85$ Hz imidazole C_2–H), 11.90 (H, s, NH–CO). EI-MS (*m*/*z*): M + 1: 480.

*4.1.5. General procedure for 2-(4-substitutedpiperazine-1-yl)-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide derivatives (**16**–**23**)*

A mixture of **6** (0.803 g, 2 mmol), corresponding 1-substituted piperazine derivative (3 mmol) and K_2CO_3 (0.276 g, 2 mmol) in acetone (30 mL) was refluxed for 24 h. After cooling, the solution was evaporated until dryness. The oily residue was treated with 5 mL of ether. Solidified product was filtered, washed with water and recrystallized from ethanol to give the **16**–**23**.

*4.1.5.1. 2-(4-Methylpiperazine-1-yl)-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**16**).* Yield 69%. m.p. 122 °C. IR (KBr) ν_{maks}(cm^{-1}): 3241 (N–H), 1697 (C=O), 1603–1408 (C=C and C=N), 847 (1,4-disubstituted benzene), 781–700 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.18 (3H, s, piperazine N–CH_3), 2.40 (4H, br, piperazine $C_{3,5}$–H), 2.54 (4H, br, piperazine $C_{2,6}$–H), 3.16 (2H, s, CO–CH_2), 3.48 (3H, s, imidazole N–CH_3), 7.12–7.24 (3H, m, C_6H_5–H), 7.42–7.57 (7H, m, C_6H_5–H), 7.74 (2H, d, $J = 8.65$ Hz, C_6H_4, $C_{2,6}$–H), 7.82 (2H, d, $J = 8.61$ Hz, C_6H_4, $C_{3,5}$–H), 9.91 (H, br, NH–CO). EI-MS (*m*/*z*): M + 1: 466.

*4.1.5.2. 2-(4-Ethylpiperazine-1-yl)-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**17**).* Yield 74%. m.p. 171 °C. IR (KBr) ν_{maks}(cm^{-1}): 3295 (N–H), 1684 (C=O), 1593–1395 (C=C and C=N), 839 (1,4-disubstituted benzene), 774–698 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 1.01 (3H, t, $J = 7.14$ Hz and $J = 7.20$ Hz, $-CH_2\underline{CH_3}$), 2.34 (2H, q, $J = 7.17$ Hz and $J = 7.18$ Hz, $-\underline{CH_2}CH_3$), 2.44 (4H, br, piperazine $C_{3,5}$–H), 2.56 (4H, br, piperazine $C_{2,6}$–H), 3.15 (2H, s, CO–CH_2), 3.48 (3H, s, imidazole N–CH_3), 7.12–7.24 (3H, m, C_6H_5–H), 7.42–7.57 (7H, m, C_6H_5–H), 7.74 (2H, d, $J = 8.70$ Hz, C_6H_4, $C_{2,6}$–H), 7.82 (2H, d, $J = 8.69$ Hz, C_6H_4, $C_{3,5}$–H), 9.91 (H, br, NH–CO). EI-MS (*m*/*z*): M + 1: 480.

*4.1.5.3. 2-[4-(2-Dimetylaminoethyl)piperazine-1-yl]-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**18**).* Yield 61%. m.p. 130 °C. IR (KBr) ν_{maks}(cm^{-1}): 3236 (N–H), 1694 (C=O), 1603–1400 (C=C and C=N), 853 (1,4-disubstituted benzene), 781–700 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.14 (6H, s, $-N(CH_3)_2$), 2.33 (4H, m, $-CH_2CH_2$), 2.48 (4H, br, piperazine $C_{3,5}$–H), 2.54 (4H, br, piperazine $C_{2,6}$–H), 3.15 (2H, s, CO–CH_2), 3.48 (3H, s, imidazole N–CH_3), 7.12–7.24 (3H, m, C_6H_5–H), 7.42–7.57 (7H, m, C_6H_5–H), 7.74 (2H, d, $J = 8.55$ Hz, C_6H_4, $C_{2,6}$–H), 7.82 (2H, d, $J = 8.53$ Hz, C_6H_4, $C_{3,5}$–H), 9.91 (H, br, $\underline{NH}$–CO). EI-MS (*m*/*z*): M + 1: 523.

*4.1.5.4. 2-(4-Phenylpiperazine-1-yl)-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**19**).* Yield 79%. m.p. 227 °C. IR (KBr) ν_{maks}(cm^{-1}): 3281 (N–H), 1676 (C=O), 1597–1397 (C=C and C=N), 856 (1,4-disubstituted benzene), 796–698 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.71 (4H, t, $J = 4.45$ Hz and $J = 4.57$ Hz, piperazine $C_{2,6}$–H), 3.23 (4H, t, $J = 4.27$ Hz and $J = 4.76$ Hz, piperazine $C_{3,5}$–H), 3.25 (2H, s, CO–CH_2), 3.48 (3H, s, imidazole N–CH_3), 6.79 (H, t, $J = 7.23$ Hz and $J = 7.34$ Hz, >N–C_6H_5, C_4–H), 6.97 (2H, d, $J = 8.38$ Hz, >N–C_6H_5, $C_{2,6}$–H), 7.12–7.25 (5H, m, C_6H_5–H and >N–C_6H_5, $C_{3,5}$–H), 7.42–7.57 (7H, m, C_6H_5–H), 7.75 (2H, d, $J = 8.37$ Hz, C_6H_4, $C_{2,6}$–H), 7.84 (2H, d, $J = 8.49$ Hz, C_6H_4, $C_{3,5}$–H), 10.00 (H, br, NH–CO). EI-MS (*m*/*z*): M + 1: 528.

*4.1.5.5. 2-(4-Benzylpiperazine-1-yl)-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**20**).* Yield 73%. m.p. 206 °C. IR (KBr) ν_{maks}(cm^{-1}): 3331 (N–H), 1688 (C=O), 1595–1400 (C=C and C=N), 839 (1,4-disubstituted benzene), 781–696 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.47 (4H, br, piperazine $C_{2,6}$–H), 2.56 (4H, br, piperazine $C_{3,5}$–H), 3.17 (2H, s, CO–CH_2), 3.48 (3H, s, imidazole N–CH_3), 3.50 (2H, s, $C_6H_5-\underline{CH_2}$), 7.13–7.25 (8H, m, C_6H_5–H and $\underline{C_6H_5}-CH_2$), 7.42–7.57 (7H, m, C_6H_5–H), 7.74 (2H, d, $J = 8.70$ Hz, C_6H_4, $C_{2,6}$–H), 7.81 (2H, d, $J = 8.73$ Hz, C_6H_4, $C_{3,5}$–H), 9.90 (H, br, NH–CO). EI-MS (*m*/*z*): M + 1: 542.

*4.1.5.6. 2-[4-(4-Methoxybenzyl)piperazine-1-yl]-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**21**).* Yield 75%. m.p. 192 °C. IR (KBr) ν_{maks}(cm^{-1}): 3378 (N–H), 1684 (C=O), 1599–1397 (C=C and C=N), 841 (1,4-disubstituted benzene), 785–706 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ(ppm): 2.43 (4H, br, piperazine $C_{2,6}$–H), 2.55 (4H, br, piperazine $C_{3,5}$–H), 3.17 (2H, s, CO–CH_2), 3.42 (2H, s, $C_6H_5-\underline{CH_2}$), 3.48 (3H, s, imidazole N–CH_3), 3.74 (3H, s, O–CH_3), 6.89 (2H, d, $J = 8.58$ Hz, $\underline{C_6H_4}$–OCH_3 $C_{3,5}$–H), 7.12–7.23 (5H, m, C_6H_5–H and $-\underline{C_6H_4}$–OCH_3 $C_{2,6}$–H), 7.42–7.57 (7H, m, C_6H_5–H), 7.74 (2H, d, $J = 8.64$ Hz, C_6H_4, $C_{2,6}$–H), 7.81 (2H, d, $J = 8.67$ Hz, C_6H_4, $C_{3,5}$–H), 9.90 (H, br, NH–CO). EI-MS (*m*/*z*): M + 1: 572.

*4.1.5.7. 2-[4-(4-Methylbenzyl)piperazine-1-yl]-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**22**).* Yield 71%. m.p. 198 °C. IR (KBr) ν_{maks}(cm^{-1}): 3241 (N–H), 1694 (C=O), 1605–1406 (C=C and C=N), 835 (1,4-disubstituted benzene), 781–698 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.28 (3H, s, $-CH_3$), 2.44 (4H, br, piperazine $C_{2,6}$–H), 2.55 (4H, br, piperazine $C_{3,5}$–H), 3.16 (2H, s, CO–CH_2), 3.43 (2H, s, $C_6H_5-\underline{CH_2}$), 3.48 (3H, s, imidazole N–CH_3), 6.89 (2H, d, $J = 8.58$ Hz, $\underline{C_6H_4}$–CH_3 $C_{3,5}$–H), 7.12–7.24 (7H, m, C_6H_5–H and $-\underline{C_6H_4}$–CH_3), 7.42–7.56 (7H, m, C_6H_5–H), 7.74 (2H, d, $J = 8.68$ Hz, C_6H_4, $C_{2,6}$–H), 7.81 (2H, d, $J = 8.68$ Hz, C_6H_4, $C_{3,5}$–H), 9.98 (H, br, NH–CO). EI-MS (*m*/*z*): M + 1: 556.

*4.1.5.8. 2-[4-(4-Chlorobenzyl)piperazine-1-yl]-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide (**23**).* Yield 82%. m.p. 196 °C. IR (KBr) ν_{maks}(cm^{-1}): 3331 (N–H), 1688 (C=O), 1593–1402 (C=C and C=N), 843 (1,4-disubstituted benzene), 787–698 (monosubstituted benzene). ^{1}H NMR (500 MHz) (DMSO-d_6) δ (ppm): 2.46 (4H, br, piperazine $C_{2,6}$–H), 2.56 (4H, br, piperazine $C_{3,5}$–H), 3.17 (2H, s, CO–CH_2), 3.47 (3H, s, imidazole N–CH_3), 3.49 (2H, s, C_6H_5–$\underline{CH_2}$), 7.12–7.24 (3H, m, C_6H_5–H), 7.34 (2H, d, J = 8.44 Hz, 4-Cl–C_6H_4, $C_{2,6}$–H), 7.39 (2H, d, J = 8.43 Hz, 4-Cl–C_6H_4, $C_{3,5}$–H), 7.42–7.56 (7H, m, C_6H_5–H), 7.74 (2H, d, J = 8.68 Hz, C_6H_4, $C_{2,6}$–H), 7.81 (2H, d, J = 8.71 Hz, C_6H_4, $C_{3,5}$–H), 9.90 (H, br, NH–CO). EI-MS (m/z): M + 1: 576.

4.2. Biochemistry

4.2.1. Cell cultures

MCF-7 cells were maintained in 90% Dulbecco's modified Eagle's medium (DMEM) (Sigma, Deisenhofen, Germany), 1 mM sodium pyruvate (Sigma, Deisenhofen, Germany), 10 μg/mL human insulin (Sigma, Deisenhofen, Germany) and 10% (v/v) of fetal bovine serum (FBS) (Gibco, U.K.). HT-29 cells were cultured in 90% McCoy's 5A (Sigma, Deisenhofen, Germany) and 10% fetal bovine serum (FBS) (Gibco, U.K.). All media were supplemented with penicillin/streptomycin at 100 U/mL and cells were incubated at 37 °C in a 5% CO_2/95% air humidified atmosphere.

4.2.2. MTT assay

A tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), was used as a colorimetric substrate for measuring cytotoxicity (MTT assay). The assay was carried out according to previous study [35]. Briefly, HT-29 and MCF-7 cells were cultured in 96 well plates and 0.64–80 μg/ml imidazole-(benz)azoles, imidazole–piperazines or cisplatin were added. The plates were incubated for 24 h at 37 °C in 5% CO_2 humidified incubator together with untreated control sample. After the incubation period, 20 μl MTT dye was added and the plates were measured with an ELx808-IU Bio-Tek apparatus at 540 nm. Control cell viability was regarded as 100%. Stock solutions of the test compounds were dissolved in DMSO and further concentrations were prepared in cell culture media. All experiments were repeated three times. Four independent wells were used for each of the compound concentration. Percent viability was defined as the relative absorbance of treated versus untreated control cells.

4.2.3. DNA synthesis inhibition assay

This method was performed in the 96 well flat-bottomed microtiter plates by using a BrdU colorimetric kit. HT-29 and MCF-7 cells were collected from cell cultures by 0.25% trypsin/EDTA solution and counted in a hemocytometer. Suspensions of cell lines were seeded into 96-well flat-bottomed microtiter plates at a density of 1×10^3 cells/mL. The tumor cell lines were cultured in the presence of various doses of the test compounds or cisplatin. Microtiter plates were incubated at 37 °C in a 5% CO_2/95% air humidified atmosphere for 24 h and 48 h. At the end of each day, the cells were labelled with 10 μl BrdU solution for 2 h and then fixed. Anti-BrdU-POD (100 μl) was added and incubated for 90 min. Finally, microtiter plates were washed with phosphate buffer saline (PBS) three times and the cells were incubated with substrate solution until the colour was sufficient for photometric detection. Absorbance of the samples was measured with an ELx808-IU Bio-Tek apparatus at 492 nm. As a control solvent, DMSO was added to the cells during the time course. The values of blank wells were subtracted from each well of treated and control cells. The absorbance values of background control wells did not exceed 0.1. All experiments were repeated three times. For each concentration of the compounds, triplicate wells were used.

4.2.4. Detection of apoptotic DNA ladder

HT-29 cells (1×10^6 cells/mL) were harvested into dishes and incubated with various doses of the test compounds for 24 h. After the incubation period, cells were washed in PBS, tripsynezed, put into tubes and then mixed. Binding buffer (200 μl) was added and incubated at R.T. for 10 min then isopropanol (100 μl) was added and mixed. The mixture was filtrated and centrifuged at 8000 rpm. Washing buffer (500 μl) was added and centrifuged again at the same rpm. Filter tubes were solubilisated with elution buffer and analyzed by 1% agarose gel electrophoresis (containing 500 μg/mL of ethidium bromide) at 50V for 120 min. Approximately 20 μg of DNA was loaded in each well, visualized under a UV light and photographed (Fig. 3).

4.2.5. Statistics

The SPSS for Windows 11.5 computer program was used for statistical analyses. Statistical comparison of the results obtained from controls, groups, and time periods parameters were carried out by the one-way analyses of variance (ANOVA) test and post hoc analyses of group differences were performed by the Tukey test. Results were expressed as mean ± SD.

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