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Novel 3-benzylbenzo[*d*]thiazol-2(3*H*)-iminium salts as potent DNA benzylating agents: design, synthesis, MTT assay, and DFT calculation

Abstract. New derivatives of 3-benzylbenzo[*d*]thiazol-2(3*H*)-iminium salt were developed, synthesized, and assessed for their cytotoxic effects on the MCF-7 cell line. Among them, two molecules, identified as 3g and 3i, demonstrated notable cytotoxic activity with IC_{50} values of 41.76 and 58.34 µmol/L respectively, compared to the reference drug Cis-platin, which has an IC_{50} of 22.36 ± 2.98 µmol/L. Subsequent DNA interaction docking studies were conducted with these compounds. The docking data revealed that both 3g and 3i effectively bind within the minor groove of DNA, showing a preference for interaction with AT-rich sequences over CG-rich sequences. These findings suggest that 3g and 3i are effective DNA-binding agents. Further analysis using Density Functional Theory (DFT) was performed to explore the potential of DNA benzylation by compound 3g. The DFT studies suggested that the benzylation of guanine bases by 3g could occur at room temperature. Nonetheless, further experimental investigations are necessary to validate this hypothesis. The DFT studies suggested that the benzylation of guanine bases by 3g could occur at room temperature. Nonetheless, further experimental investigations are necessary to validate this hypothesis.
Key words: 3-Benzylbenzo[*d*]thiazol-2(3*H*)-iminium salt, cytotoxic, DNA interaction, cancer, 2-Aminobenzothiazol.

Introduction

Benzothiazole (BTA) is a bicyclic heterocycle consisting of a 1,3-thiazole ring attached to a benzene ring. BTA is found in many natural products and is responsible for many medicinal and pharmacological properties [1]. BTA scaffold has a wide range of biological activities such as antidiabetic [2], fungicidal [3], antileishmanial [4], anti-Alzheimer [5], anticonvulsants [6], antituberculosis [7], antibacterial [8], and anthelmintic [9], etc., schematically presented on Figure 1.

Also, BTA derivatives exhibit prevalent and remarkable cytotoxic effects against some types of tumors and cancer cell lines e.g. human colon adenocarcinoma cell line (SW480), human cervical cancer cell line (HeLa), human liver carcinoma cells (HepG2) [10], nonsmall-cell lung, colon [11], human breast cancer cell line (MCF-7) [12], and hepatocellular carcinoma (HCC) [13] and so on. The sentences are only a very small part of the biological applications of BTA derivatives. Therefore, researchers are very interested in synthesizing new derivatives based on BTA and evaluating their medicinal properties.

DNA benzylating agent can be defined as a compound capable of covalently attaching a benzyl group to the DNA nucleobases under the physiological conditions. In this reaction, compounds always behave as carbon electrophiles and some nitrogen or oxygen atoms of DNA nucleobases are nucleophiles [14, 15]. Both structure and dynamics of DNA are seriously changed by benzylation of its nucleobases. As a result, DNA strand undergoes structural deformations that affect both replication and/or transcription stages. Benzylation also induces the mispairing of the nucleobases by change of the natural hydrogen bonding between the bases. Therefore, benzylating agents can destroy cancer cells by damaging their DNA [16].

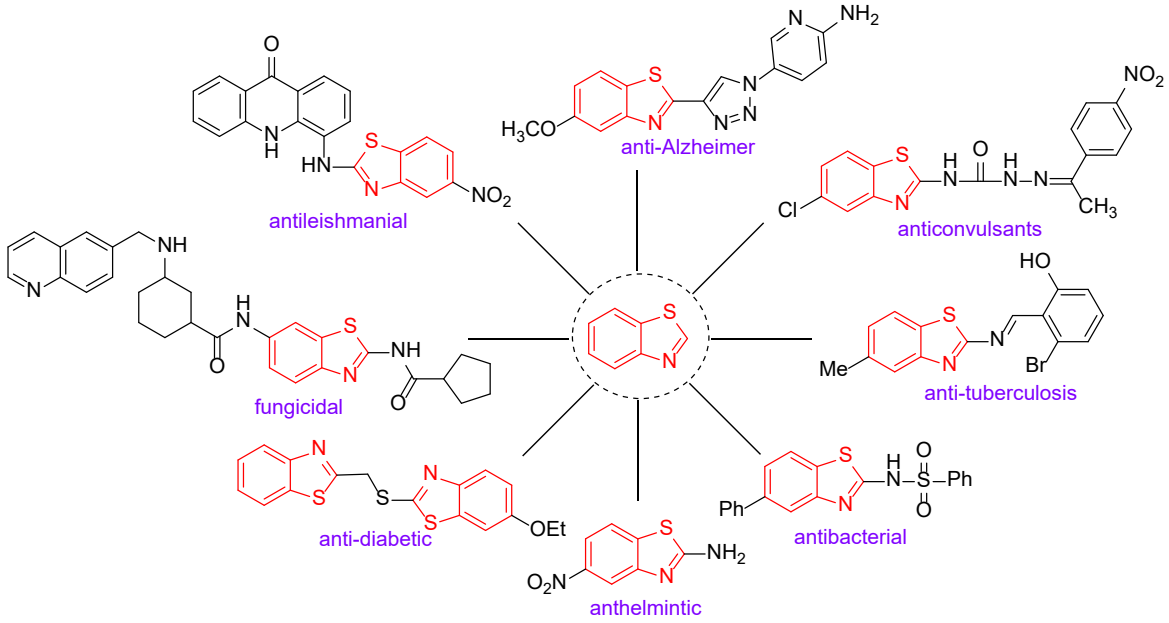


Figure 1 – Some biologically active compounds with benzothiazole scaffold

In continuation of our investigations on biologically active heterocyclic compounds [17-20], mainly the synthesis of cytotoxic compounds [21-23], new 3-benzylbenzo[*d*]thiazol-2(3*H*)-iminium salt derivatives are synthesized, characterized and evaluated for their cytotoxic activities.

Materials and methods

Chemistry

Synthesis of 3-benzylbenzo[d]thiazol-2(3H)-iminium salts*: *General procedure

In a 50 ml round bottom flask, a solution was prepared by dissolving 13.7 mmol of 2-aminobenzothiazole derivatives and 18.2 mmol of benzyl halide derivatives in 25 ml of n-butanol, to which one mmol of sodium iodide was subsequently added. This mixture was then subjected to reflux for a duration of 8 hours. Monitoring of the reaction's progress was conducted using thin layer chromatography (TLC) employing a solvent system composed of equal parts *n*-hexane and ethyl acetate. Following the reaction, the mixture was filtered to collect any solids, which were then washed with water and crystallized using methanol.

Spectral data were collected for the resulting products:

***3a**: 3-Benzyl Benzo [d] Thiazol-2(3H)-Iminium Bromide*

^{1}H NMR (301 MHz, DMSO d_6) δ: 10.56 (2H, s), 8.06 (1H, d, J = 8.1 Hz), 7.55 (1H, d, J = 8.0 Hz), 7.26-7.38 (5H, d, J = 7.2 Hz), 5.66 (d, J = 7.9 Hz, 2H), 3.16 (2H, s). IR-KBr(cm^{-1}) v: 3263, 3078, 1663, 1558 and 1470; MS m/z (%): 320.0.

***3b**: 3-(4-Fluorobenzyl) Benzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) δ: 11.21 (2H, s), 8.04 (1H, d, J = 7.9 Hz), 7.60 (1H, d, J = 8.0 Hz), 7.18-7.42 (4H, d, J = 7.5 Hz), 5.66 (2H, d, J = 7.9 Hz), 3.16 (2H, s). IR-KBr(cm^{-1}) v: 1650, 1566.85 and 1472; MS m/z (%): 294.0.

***3c**: 3-(4-Chlorobenzyl) Benzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) δ: 11.06 (2H, s), 8.02 (1H, d, J = 8.1 Hz), 7.57 (1H, d, J = 8.0 Hz), 7.30-7.49 (4H, d, J = 7.2 Hz), 5.74 (2H, d, J = 7.9 Hz), 3.42 (2H, s). IR-KBr(cm^{-1}) v: 1644, 1564 and 1471; MS m/z (%): 310.0.

***3d**: 3-(4-Methylbenzyl) Benzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) δ: 11.39 (2H, s), 8.03 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 7.7 Hz, 1H), 7.31-7.51 (d, J = 7.4 Hz, 4H), 7.17 (d, J = 7.7 Hz, 2H), 3.40 (2H, s), 2.30 (3H, s). IR-KBr(cm^{-1}) v: 3293, 1644, 1566 and 1469; MS m/z (%): 290.1.

***3e**: 3-Benzyl-5-Methylbenzo [d] Thiazol-2(3H)-Iminium Bromide*

^{1}H NMR (301 MHz, DMSO d_6) δ: 10.39 (2H, s), 7.84 (1H, d, J = 7.7 Hz), 7.46 (1H, d, J = 7.7 Hz), 7.25-7.40 (5H, d, J = 7.3 Hz), 5.61 (2H, d, J = 7.7 Hz), 3.20 (2H, s), 2.42 (3H, s). IR-KBr(cm^{-1}) v: 3457, 1632, 1566 and 1487; MS m/z (%): 334.2.

***3f**: 3-(4-Fluorobenzyl)-5-Methylbenzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) *δ*: 11.06 (2H, s), 7.83 (1H, d, *J* = 7.4 Hz), 7.51 (1H, d, *J* = 7.5 Hz), 7.18-7.42 (5H, d, *J* = 7.2 Hz), 5.75 (2H, d, *J* = 7.4 Hz), 4.02 (2H, s), 2.52 (3H, s). IR-KBr(cm^{-1}) *v*: 1637, 1566 and 1454; MS m/z (%): 308.2.

***3g**: 3-(4-Chlorobenzyl)-5-Methylbenzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) *δ*: 11.01 (2H, s), 7.82 (1H, d, *J* = 7.5 Hz), 7.45 (1H, d, *J* = 7.4 Hz), 7.28-7.42 (4H, d, *J* = 7.4 Hz), 5.79 (2H, d, *J* = 7.6 Hz), 3.40 (2H, s), 2.52 (3H, s). IR-KBr(cm^{-1}) *v*: 1650, 1568 and 1490; MS m/z (%): 324.0.

***3h**: 5-Methyl-3-(4-Methylbenzyl) Benzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) *δ*: 11.01 (2H, s), 7.81 (1H, d, *J* = 7.4 Hz), 7.43 (1H, d, *J* = 7.4 Hz), 7.31-7.16 (4H, d, *J* = 7.3 Hz), 5.67 (2H, d, *J* = 7.4 Hz), 3.38 (2H, s), 2.51 (6H, s). IR-KBr(cm^{-1}) *v*: 1647, 1574 and 1488; MS m/z (%): 304.1.

***3i**: 3-Benzyl-5-Ethoxybenzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) *δ*: 10.41 (2H, s), 7.76 (1H, d, *J* = 7.5 Hz), 7.49 (1H, d, *J* = 7.4 Hz), 7.03-7.39 (5H, d, *J* = 7.4 Hz), 5.66 (2H, d, *J* = 7.5 Hz), 4.01 (2H, s), 2.53 (3H, t), 1.31 (2H, m). MS m/z (%): 320.

***3j**: 5-Ethoxy-3-(4-Fluorobenzyl) Benzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) *δ*: 10.87 (2H, s), 7.88 (1H, d, *J* = 7.4 Hz), 7.48 (1H, d, *J* = 7.5 Hz), 7.04-7.37 (4H, d, *J* = 7.7 Hz), 5.69 (2H, d, *J* = 7.3 Hz), 4.03 (2H, s), 2.00 (3H, t), 1.31 (2H, m). IR-KBr(cm^{-1}) *v*: 1644, 1576 and 1490; MS m/z (%): 338.2.

***3k**: 5-Ethoxy-3-(4-Methylbenzyl) Benzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) *δ*: 10.89 (2H, s), 7.68 (1H, d, *J* = 7.2 Hz), 7.47 (1H, d, *J* = 7.3 Hz), 7.02-7.23 (4H, d, *J* = 7.3 Hz), 5.66 (2H, d, *J* = 7.2 Hz), 4.02 (2H, s), 2.30 (t, 6H), 1.33 (2H, m). IR-KBr(cm^{-1}) *v*: 1648, 1563 and 1488; MS m/z (%): 334.

***3l**: 3-(4-Chlorobenzyl)-5-Ethoxybenzo [d] Thiazol-2(3H)-Iminium Chloride*

^{1}H NMR (301 MHz, DMSO d_6) *δ*: 10.98 (2H, s), 7.67 (1H, d, *J* = 7.4 Hz), 7.47 (1H, d, *J* = 7.4 Hz), 7.03-7.36 (4H, d, *J* = 7.2 Hz), 5.72 (2H, d, *J* = 7.3 Hz), 4.03 (2H, s), 2.53 (t, 6H), 1.34 (2H, m).

MTT assay

Studies on cell viability were conducted using the MCF-7 breast cancer cell line. These cells were cultured in DMEM high glucose medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin, maintained in an environment of 95% humidity and 5% CO_2. The viability of the cells was assessed using an MTT assay. Initially, 1.0×10^4 MCF-7 cells were seeded in each well of a 96-well plate and allowed to incubate for 16 hours. Various concentrations of compounds (**3a-l**) ranging from 12.5 to 100 μM were then exposed to the cells for a duration of 72 hours. Subsequently, each well received an MTT solution at a concentration of 0.50 mg/ml, followed by an additional incubation period of 4 hours under the same conditions. After this period, the culture medium was removed, and the formazan precipitate was dissolved in 100 μL of pure DMSO. The absorbance was measured using a BMG Spectro Nano Elizabeth Reader at wavelengths of 570 nm and 630 nm, which correspond to formazan and background absorbance respectively. Cell viability was quantified using the formula: Cell viability % = [AT (sample) / AT (control)] × 100, where AT represents the adjusted absorbance (A570 – A30).

The mean viability percentage along with the standard deviation was calculated from three independent experiments. The IC_{50} values were calculated using GraphPad Prism version 9.0 software.

Molecular docking studies

Docking simulations were accomplished by AutoDock 4.2 software according to the conditions mentioned in our previously reported paper [22].

DFT calculation

Density functional theory (DFT) calculations were done using the ORCA quantum chemistry package [24]. The structure of compound 3g, guanine, and Chlormethine were optimized with the BP86 functional applying RI approximation and a TZVP basis set together with a matching auxiliary basis set (TZV/J). Numerical frequency was calculated at the same level. Initial guess of the transition state (TS) was based on the S_N2 mechanism. Single point energy calculations were done with the PW6B95 method by applying Def2-TZVP basis set.

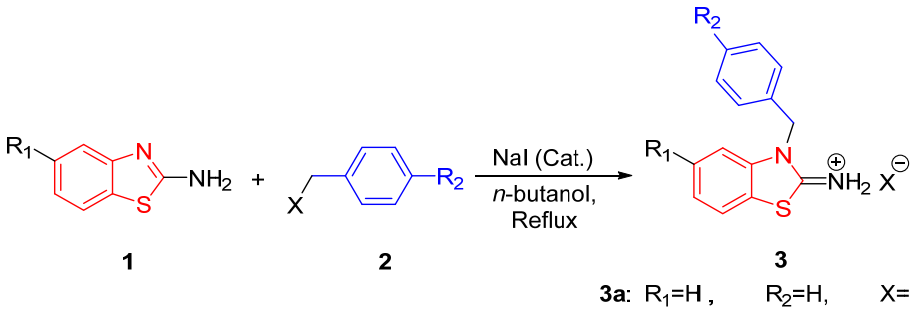
Results and discussion

Chemistry

The preparation reaction of 3-benzylbenzo[*d*] thiazol-2(3*H*)-iminium salt derivatives (**3a-l**) is shown on Figure 2. In the presence of NaI as catalyst, various 2-aminobenzothiazoles (1) and benzyl halides (2) react under the reflux of *n*-butanol to obtain the desired products **3a-l** (moderate to good yields).

New 3-benzylbenzo[*d*]thiazol-2(3*H*)-iminium salts were characterized by IR, ^{1}H NMR, and mass spectroscopy. Figure 3 presents the ^{1}H NMR spectrum of 3-benzyl-5-ethoxybenzo[*d*]thiazol-2(3*H*)-iminium bromide (**3i**) as a model compound. A triplet peak at 1.29-1.34 ppm corresponds to the -CH_3 group and a quartet peak at 3.99-4.06 ppm belongs to the CH_2 of ethyl group. The peak related to benzylic CH_2 appeared in the region of 5.66 ppm as a singlet and with integral 2. The peaks of the aromatic protons appeared in 7.3 to 7.76 ppm as multiplets and with integral 8. The peak related to NH_2 protons appeared in the region of 10.41 ppm as a broad peak with integral 2. The use of D_2O solvent during spectroscopy caused the peak of this region to be removed, which proves that these peaks are related to hydrogens attached to N.

Although we expected the benzyl group to be attached to NH_2, this group was attached to the nitrogen of the benzothiazole ring. Products out of salt form were nowhere to be found.



3a: R_1=H , R_2=H, X= Br
3b: R_1=H , R_2=F, X= Cl
3c: R_1=H , R_2=Cl, X= Cl
3d: R_1=H , R_2=CH_3, X= Cl
3e: R_1=CH_3 , R_2=H, X= Br
3f: R_1=CH_3 , R_2=F, X= Cl
3g: R_1=CH_3 , R_2=Cl, X= Cl
3h: R_1=CH_3 , R_2=CH_3, X= Cl
3i: R_1=OEt , R_2=H, X= Br
3j: R_1=OEt , R_2=F, X= Cl
3k: R_1=OEt , R_2=CH_3, X= Cl
3l: R_1=OEt , R_2=Cl, X= Cl

Figure 2 – Synthesis of 3-benzylbenzo[*d*]thiazol-2(3*H*)-iminium salt derivatives (3a-l)

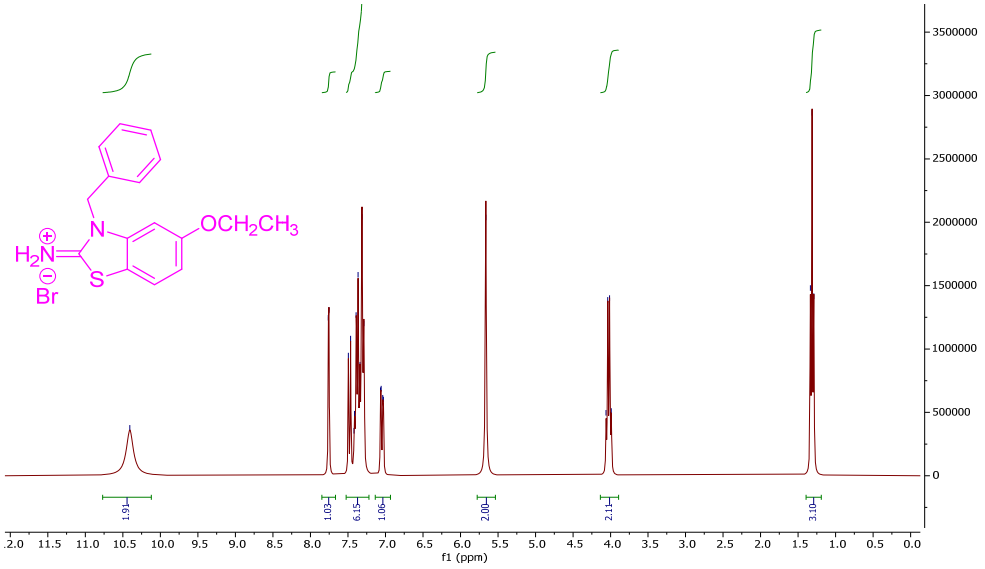


Figure 3 – ^{1}H NMR spectrum of 3-benzyl-5-ethoxybenzo[*d*]thiazol-2(3*H*)-iminium bromide (**3i**)

MTT assay

Table 1 details our findings on the antitumor efficacy of derivatives **3a-3l** of 3-benzylbenzo[*d*] thiazol-2(3*H*)-iminium salt against the MCF-7 human breast cancer cell line. Among these, only compounds **3g** and **3i** demonstrated notable activity, with IC_{50} values of 41.76 µmol/L and 58.34 µmol/L respectively, when compared to the reference anticancer drug Cis-platin, which has an IC_{50} value of 22.36 µmol/L. Specifically, the compound 3-(4-chlorobenzyl)-5-methylbenzo[d]thiazol-2(3*H*)-iminium chloride (**3g**), featuring a chlorine atom at the 4 position on the benzyl and a methyl group at the 5 position of the benzothiazole ring, showed a greater antiproliferative effect than 3-benzyl-5-ethoxybenzo[d]thiazol-2(3H)-iminium bromide (**3i**). The other derivatives did not exhibit significant cytotoxic effects, making it impractical to assess the impact of different substituents on the benzothiazole ring.

Molecular modeling studies

In silico study of the ligand-DNA interaction is one of the most essential aspects of medicinal chemistry researches with the aim of discovering and developing new kinds of anti-cancer drugs [25]. In this regard, we investigated the interactions of **3g** and **3i** with DNA.

Using docking software, we studied the binding of **3g** and **3i** with two DNA chains, 1CGC [decamer of the repeated Cytosine-Guanine d(CCGGCGCCGG)] and 1DNE [dodecamer of two repeats of Adenine-Thymine d(CGCGATATCGCG)] [26]. The results are shown in Table 2. These compounds interacted with the minor groove of DNA. Each amino group of these compounds had a hydrogen bond with the ribose ring of the DNA. The benzylic group was placed along the minor groove and participated in van der Waals interactions.

Table 3 shows the free energy DNA-binding values (ΔG) of the ligands. **3g** interacted more effectively than **3i** with the minor groove and penetrated the groove better. The affinity of both compounds to the AT-rich chain was higher than to the CG-rich chain. We compared the free energy binding values of **3g** and **3i** with pyriproxyfen [22]. The results showed that **3g** binds to 1DNA better than others. However, pyriproxyfen establishes a better binding with 1CGC than **3g** and **3i**.

Table 1 – The IC_{50} values of the complexes

Ligand	IC_{50} (µM)
3a	>100
3b	>100
3c	>100
3d	>100
3e	>100
3f	>100
3g	41.76 ± 2.26
3h	>100
3i	58.34 ± 2.59
3j	>100
3k	>100
3l	>100
Cis-platin	22.36 ± 2.98

Based on the results of docking, we concluded that 3-benzylbenzo[*d*]thiazol-2(3*H*)-iminium salt derivatives have the capability to form a stable ligand-DNA complex. Because of the positive charge, the hydrophilicities of the ligands are low (ClogP less than 0.5), and it increases the probability of interaction with the DNA chain rich in negative charge. Of course, the effect of the hydrophilicity of these derivatives in passing through the cell membrane should be evaluated biologically.

Table 2 – 3D and 2D interactions of two **3g** and **3i** with CG and AT-rich DNA chains

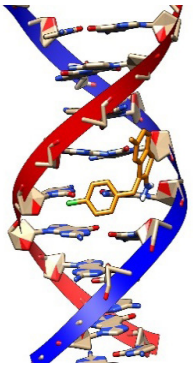
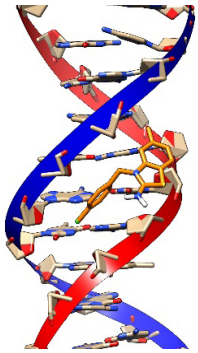
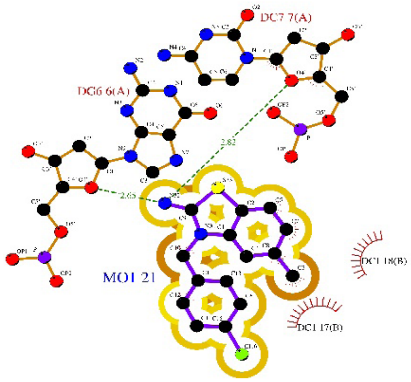
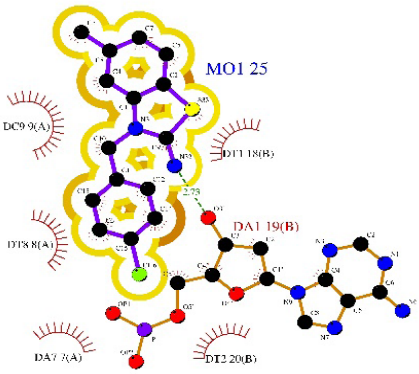
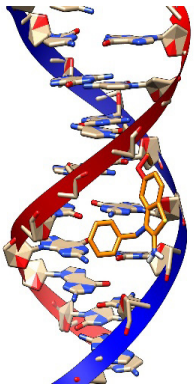
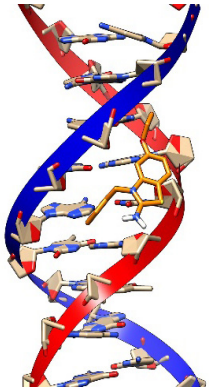
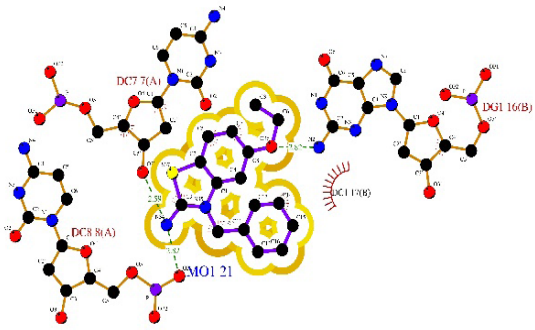
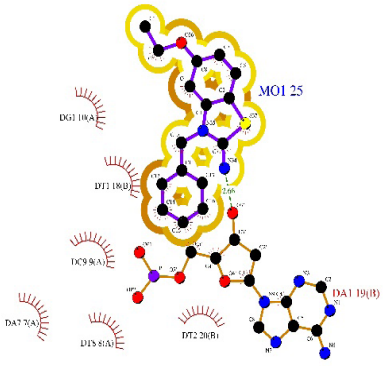
Ligand	1CGC	1DNE
3g		
		
3i		
		

Table 3 – Free energy (Kcal mol^{-1}) DNA-binding of **3g, 3i** and pyriproxyfen

Ligand	Strand rich in AT (1DNE)	Strand rich in CG (1CGC)
	Free energy of binding	Free energy of binding
3g	-6.96	-5.58
3i	-6.26	-4.98
Pyriproxyfen	-6.7	-7.4

Ligand efficiency (LE) displays the ability of the ligands to interact to the receptor, which is calculated through the below equation [27]:

$$LE = \frac{\Delta G}{N}$$, N= Number of non-hydrogen atoms

Calculated LE values of **3g**, **3i** and pyriproxyfen (as a DNA-binding model) are shown in Table 4.

Table 4 – Calculated LE amounts of **3g**, **3i** and pyriproxyfen

Ligand	Strand rich in AT (1DNE)	Strand rich in CG (1CGC)
	LE	LE
3g	0.36	0.29
3i	0.31	0.25
Pyriproxyfen	0.27	0.3

The LE calculated for **3g**-1DNE is 0.36 which is more than the LEs of compounds **3i** and pyriproxyfen, but LE calculated for **3g**-1CGC and **3i**-1CGC is lower than the LE of pyriproxyfen. These results show that compound **3g** has good ability to bind to AT-rich strand of DNA. After docking, this question occurred for us considering the structure of the products, will they be able to act as DNA benzylating agents? (Figure 4).

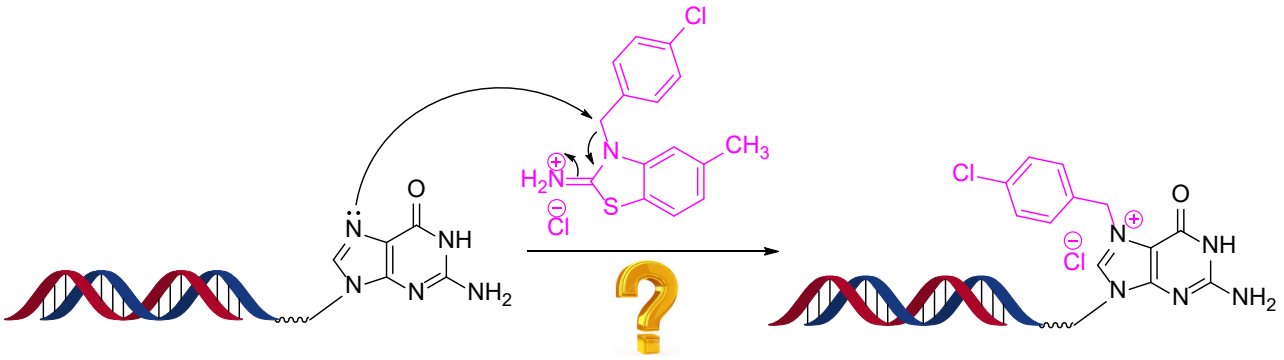


Figure 4 – Will the **3g** derivative be able to act as a DNA benzylating agent?

To answer this question, we performed DFT calculations on the compound **3g** as the most effective compound in the MTT test. DFT calculation resembled the S_N^2 attack of guanine N7 atom to benzylic carbon of compound **3g** (Figure 5).

Chlormethine, as aliphatic nitrogen mustard, was used for comparison. The transition state of the reaction was confirmed by single negative frequency. The energy of TS for the reaction of Chlormethine and guanine was 3.72 kcal/mol more stable than the starting material. The stability of transition state in comparison to starting material indicates the high reactivity of Chlormethine that is in accordance to the clinical activity of the drug. On the other hand, the TS of the compound **3g** located 18.59 kcal/mol upper than the compound and guanine. The obtained results indicated that reaction between compound 3g and guanine could take place in room temperature (activation energy less than ~20 kcal/mol) but in comparison to Chlormethine the reaction rate is slow.

Molecular study indicated that the designed compound **3g** could bind to DNA strands. Also, DFT calculation indicated that compound **3g** could alkylate the guanine in room temperature.

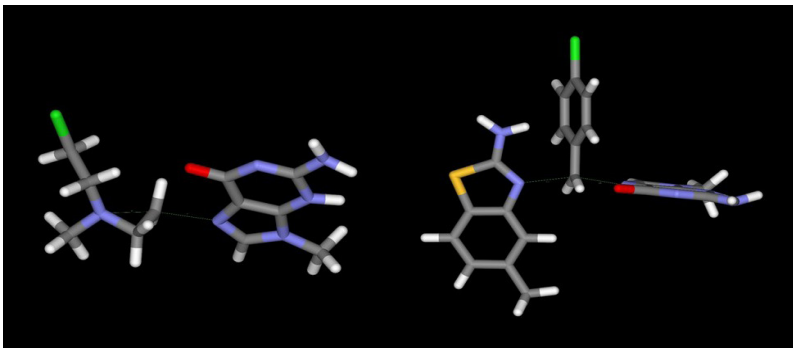


Figure 5 – TS of reaction between Chlormethine and compound 3g with guanine nucleobase.
In first case ring opening reaction leads to alkylation of guanine.
In second case, the benzylation of guanine takes places

Conclusion

We synthesized twelve new 3-benzylbenzo[*d*] thiazol-2(3*H*)-iminium salt derivatives. Then we used them in the MTT assay against the MCF-7 cell line (human breast cancer). Among all products, only two compounds **3g** and **3i** have significant cytotoxic effects against MCF-7 with IC_{50} values of 41.76 and 58.34 μmol/L respectively, compared to the Cis-platin as an anti-cancer drug (IC_{50} value 22.36 μmol/L). Then we performed molecular docking simulations on the DNA interactions of these ligands. Based on the results, these compounds can interact well with the minor groove of DNA. Of course, the interaction of the ligands with the AT-rich chain (1DNE) was better than the CG-rich chain (1CGC). Therefore, it can be concluded that **3g** and **3i** are suitable interactors with DNA. DFT calculation showed compound **3g** can benzylate the guanine base of DNA. However, Proving the DNA benzylation ability of these compounds requires experimental studies.

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Conflict of interest

All authors are aware of the article's content and declare no conflict of interest.

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