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Synthesis, Antitumor Activity, and Docking Study of 1,3-Disubstituted Imidazolium Derivatives[1]

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Abstract—A series of 1,3-disubstituted imidazolium salts were synthesized through a convenient synthetic approach based on the reaction of 1,4-diazabuta-1,3-dienes with $HClO_4$. Their antitumor activity was evaluated *in vitro* against a number of human cancer cells. 1,3-Bis[(3,5-bis(trifluoromethyl)phenyl]imidazolium perchlorate turned out to be the most active against A549 and MCF-7 cancer cell lines with IC_{50} values of 5.24 and 4.21 μM, respectively. The results of structure–activity relationship study indicated that substituents on the imidazole derivatives play an important role in their cytotoxic activities. Finally, molecular docking of some tested compounds was carried out in order to investigate their binding pattern with the CDK2.

Keywords: *N*,*N*′-substituted imidazolium salt, antitumor activity, in vitro, molecular docking

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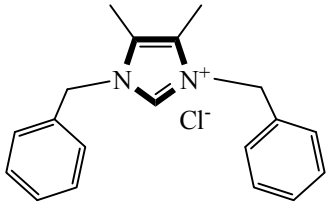
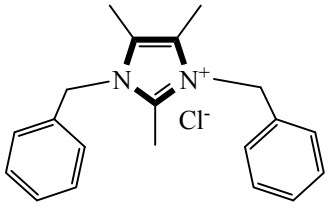
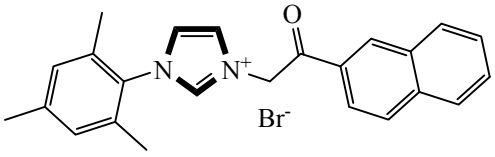
Imidazolium derivatives are important nitrogen-containing heterocyclic building blocks in developing physiologically active compounds [1–3]. To date, a considerable number of documents have been published on the synthesis of imidazolium salts and their biological and pharmacological activities, including antitumor activity [4–8]. For example, lepidilines A and B isolated from natural source exhibited potent cytotoxic activity with IC_{50} values of 1.4–7.4 μg/mL against some human cancer cell lines (UMUC3, PACA2, MDA231, and FDIGROV) [9]. The synthetic imidazolium salt MNIB demonstrated excellent antiproliferative activity with IC_{50} values of 0.3–2.2 μg/mL on various human tumor cell lines (HepG2, Raji, A549, et al.), and further molecular mechanism studies showed that it can induce cell cycle arrest and apoptosis in tumor cells [10]. In our previous paper, we have reported a convenient synthetic approach to *N*,*N*′-disubstituted imidazolium derivatives **2** and **3** through the reaction of 1,4-diazabutanenes with $HClO_4$ [11, 12]. Taking into account structural similarity of **2** and **3** to lepidilines A and B and MNIB, we evaluated the antitumor activities of **2** and **3** by MTT assay.



Lepidiline A



Lepidiline B



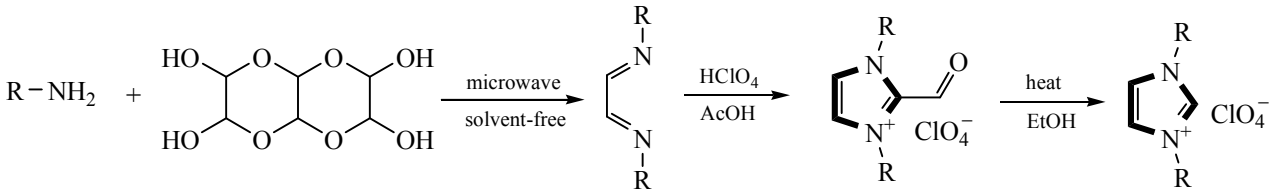
NMIB

As reported previously [11–13], the title compounds were prepared from readily accessible amines and glyoxal trimer dihydrate (hexahydro[1,4]dioxino-[2,3-*b*][1,4]dioxine-2,3,6,7-tetrol) which gave rise to 1,4-diazabutadienes **1** under solvent-free conditions with microwave assistance. Subsequently, treatment of **1** with perchloric acid in the presence of acetic acid gave the corresponding 2-formylimidazolium salts **2** which underwent decarbonylation to imidazolium derivatives **3** on heating in ethanol under reflux (Scheme 1).

In this way, ten *N*,*N*′-disubstituted imidazolium derivatives **2a**–**2c** and **3a**–**3g** were prepared in medium

[1] The text was submitted by the authors in English.

Scheme 1.



R = *i*-Pr (**a**), *t*-Bu (**b**), *cyclo*-C_6H_{11} (**c**), 2,6-$Me_2C_6H_3$ (**d**), 2,6-(*i*-Pr)$_2C_6H_3$ (**e**), 3,5-$(F_3C)_2C_6H_3$ (**f**), 2,4,6-$Me_3C_6H_2$ (**g**).

to high yields (see Experimental). The structures of **2a** and **3c** as representative compounds were determined by X-ray analysis (CCDC entry nos. 783 968, 775 890; Fig. 1).

Potential biological activity of the synthesized imidazolium derivatives was evaluated *in vitro* against a number of human tumor cell lines, namely lung carcinoma (A549), breast carcinoma (MCF-7), basal cell carcinoma (A431) and myeloid leukemia (HL-60) using MTT cytotoxicity assay. Cisplatin was chosen as a positive reference drug. The results are summarized in table.

As shown in table, almost all compounds substituted by aryl groups displayed better antitumor activities against A549, MCF-7, A431, and HL-60 cell lines than those substituted by alkyl groups. Compounds **2a**–**2c** were more active than **3a**–**3c** against all the tested cell lines, which suggested that introduction of a formyl group into the 2-position of imidazole ring enhances the inhibitory activity of imidazolium derivatives. Furthermore, among these imidazole derivatives, compound **3f** showed potent antitumor activities against A549 and MCF-7 with IC_{50} values of 5.24 and 4.21 μM, respectively.

On the other hand, it has been found that the antitumor activity decreases if the nitrogen atoms of the imidazole ring are linked to bulky groups. Thus, compounds **2a** and **2b** with bulky isopropyl and *tert*-butyl groups attached to the nitrogen atoms showed weaker activities against the tested cells in comparison to **2c**. The same tendency can also be noticed among compounds **3a**–**3c**. Moreover, comparison of the inhibitory activities of **3d**–**3g** revealed that electron-donating groups attached to the aryl group on the imidazole nitrogen atoms play a negative role in their bioactivities. For example, compound **3f** substituted by 3,5-bis(trifluoromethyl)phenyl groups, showed 4.4 to 4.5 times higher antitumor activity against A549 and MCF-7 cell lines than that of **3e**. This difference may be attributed to differences in the molecular structure, hydrophobicity, and charge distribution of imidazolium derivatives [14].

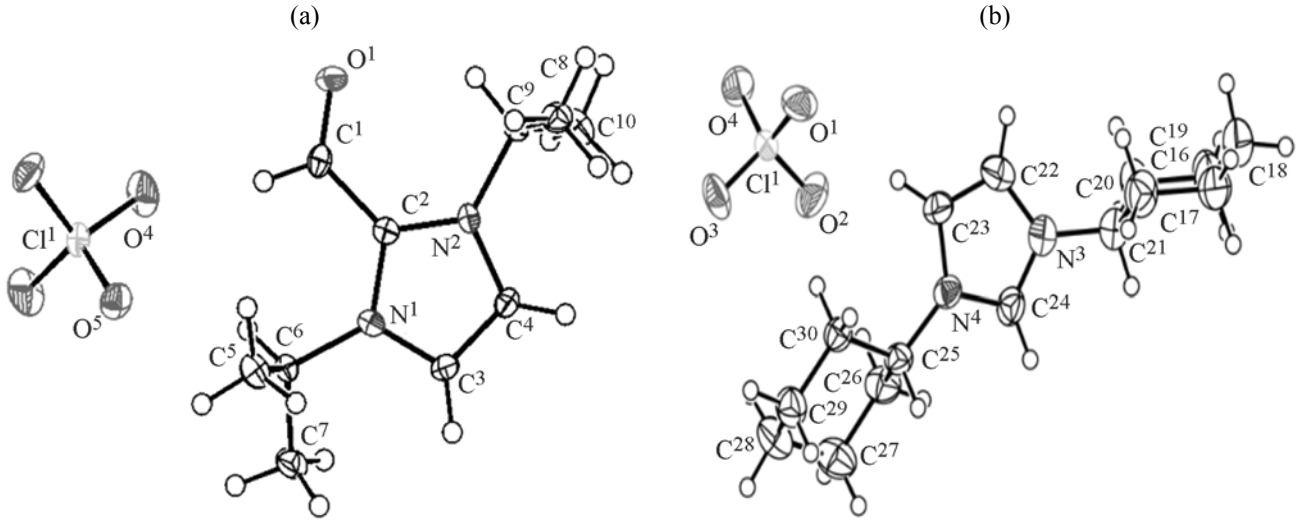


Fig. 1. X-ray crystal structures of compounds (a) **2a** and (b) **3c**.

Because of the favorable inhibitory activity of imidazolium derivative **3f** against A549 and MCF-7 cell lines, molecular docking study was carried out to explore its interaction with cyclin-dependent kinase 2 (CDK2) chosen as target protein on account of its overexpression in lung cancer and breast cancer [15–18] and elucidate differences in the bioactivities of imidazolium derivatives. We compared the structures of the complexes formed by docking of **2c**, **3f**, and **3g** with CDK2. Figure 2 shows the docking models between these compounds and CDK2 complex.

It is seen that the binding modes of **3f** and **3g** to CDK2 are identical; both ligands adopt planar conformation to fit into the protein pocket, while **2c** binds to CDK2 in a "U-type" fashion. In the docking of **3f** and **3g**, the imidazole fragments were located at the ribose pocket, while the left group attached to the imidazole ring extended into the highly hydrophobic adenine pocket, and the other is inserted into the

Antitumor activity (IC_{50},[a] μM) of compounds **2** and **3** *in vitro*

Comp. no.	A549	MCF-7	A431	HL-60
2a	28.64	22.12	45.50	47.54
2b	20.87	24.95	39.56	35.60
2c	15.24	13.21	28.33	30.15
3a	25.56	29.78	55.68	56.68
3b	31.28	32.62	52.23	54.56
3c	19.57	21.60	44.67	48.25
3d	12.64	14.12	37.50	40.54
3e	22.87	18.95	28.56	29.60
3f	5.24	4.21	15.33	17.15
3g	16.59	15.69	27.23	32.45
Cisplatin	8.36	16.25	13.67	3.46

[a] Mean values of three independent measurements.

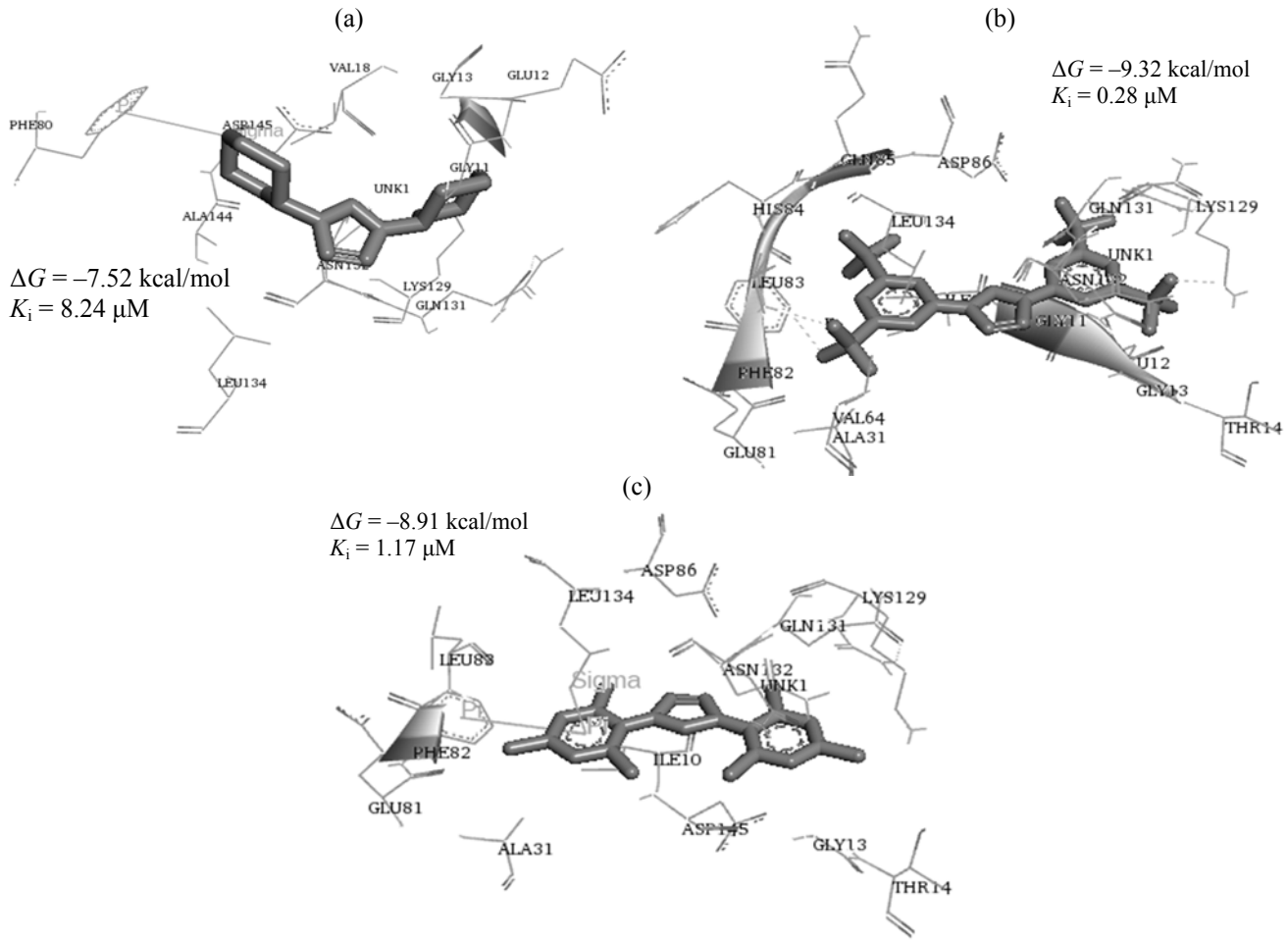


Fig. 2. Docking modes of (a) **2c**, (b) **3f**, and (c) **3g** in the ATP-binding site of CDK2.

phosphate pocket (Figs. 2b, 2c). Furthermore, the phenyl ring of **3f** (ΔG = –8.91 kcal/mol, K_i = 1.17 μM) was engaged in π–σ interaction (Ar···HC, *d* = 3.4 Å) with the Leu134 hydrophobic residue and π–π interaction (Ar···Ar, *d* = 6.2 Å) with the hinge residue Phe82. In the case of **3g** (ΔG = –9.32 kcal/mol, K_i = 0.28 μM), three hydrogen bonds were detected between the trifluoromethyl groups on the phenyl rings and the residues. Among them, two hydrogen bonds were formed by the fluorine atom acting as hydrogen bond acceptor and the amino group of the hinge residue Leu83 acting as hydrogen bond donor (CF_3···H_2N, *d* = 2.3 and 1.9 Å) and the residue Lys129 in the catalytic loop (CF_3···HN, *d* = 2.2 Å). These results suggest that hydrogen bonds have a positive effect on the interactions between the substrates and residues, which should improve the binding affinity and inhibitory activity. On the other hand, only σ–π interaction (CH···Ph, *d* = 3.9Å) between the cyclohexyl group (ΔG = –7.52 kcal/mol, K_i = 8.24 μM) and the gatekeeper residue Phe80 was involved in the complex of **2c** with CDK2 (Fig. 2a). This is likely to be responsible for the moderate antitumor activity of **2c**. On the basis of the docking models shown in Fig. 2, we speculated that bulky groups on the nitrogen atoms hinder insertion of the imidazolium derivatives into the reactive pocket of CDK2, which impairs their inhibitory activity.

In summary, we have synthesized a series of imidazolium derivatives evaluated their antitumor activities *in vitro* against A549, MCF-7, A431, and HL-60 human cancer cell lines by using MTT assay. Among the compounds rested, *N*,*N*′-bis[3,5-bis(trifluoromethyl)phenyl]imidazolium perchlorate (**3f**) turned out to be most active against A549 and MCF-7 cell lines with IC_{50} values of 5.24 μM and 4.21 μM, respectively, while the other compounds showed moderate inhibitory activities with IC_{50} values ranging from 12.64 μM to 56.68 μM. The structure–activity relationship study demonstrated that introduction of a formyl group at position *2* of the imidazole ring enhances the inhibitory activity against all the tested cancer cell lines and that imidazolium derivatives with aryl groups on the nitrogen atoms are more active than those with alkyl groups, especially with bulky alkyl groups. Molecular docking studies showed that compound **3f** interacted with CDK2 via hydrogen bonding with the highest energy score. By contrast, compounds **2c** and **3g** bind to CDK2 only through weaker van der Waals forces, such as σ–π and π–π interactions, and exhibited medium inhibitory activity. These results were well consistent with the cytotoxicity assay.

EXPERIMENTAL

All chemicals were purchased from commercial sources and used without further purification. Thin-layer chromatography (TLC) was conducted on silica gel 60 F254 plates (Merck). The melting points were determined on a XT-5A digital melting point apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 and 100 MHz, respectively, using $CDCl_3$, DMSO-d_6, or acetone-d_6 as solvent and tetramethylsilane (TMS) as internal standard. The high-resolution electrospray ionization mass spectra (ESI-MS) were measured on a ZAB-HS/Esquire 6000 mass spectrometer.

Synthesis of compounds 2a–2c *(general procedure).* A solution of $HClO_4$ in AcOH (5.0 mL, 1.0 N) was added to a solution of *N*,*N*′-disubstituted 1,4-diazabutadiene (5 mmol) in AcOH (30 mL). The mixture was stirred overnight at room temperature, and the product crystallized from the solution and was filtered off.

2-Formyl-1,3-diisopropylimidazolium perchlorate (2a). Yield 57%, mp 199–201°C. ^1H NMR spectrum, δ, ppm: 1.54 d (12H, *J* = 6.8 Hz), 5.14 sept (2H, *J* = 6.8 Hz), 8.01 s (2H), 10.11 s (1H). Mass spectrum: *m*/*z* 181.1338 $[M - ClO_4]^+$; calculated for $C_{10}H_{17}N_2O$: 181.1341.

1,3-Di-*tert*-butyl-2-formylimidazolium perchlorate (2b). Yield 61%, mp 132-133°C. ^1H NMR spectrum, δ, ppm: 1.84 s (18H), 8.11 s (2H), 10.80 s (1H). Mass spectrum: *m*/*z* 181.1642 $[M - CO - ClO_4]^+$; calculated for $C_{11}H_{21}N_2$: 181.1699.

1,3-Dicyclohexyl-2-formylimidazolium perchlorate (2c). Yield 89%, mp 282–283°C. ^1H NMR spectrum, δ, ppm: 1.43–2.07 m (20H), 4.97 m (2H), 8.28 s (2H), 10.15 s (1H). Mass spectrum: *m*/*z* 261.1940 $[M - ClO_4]^+$; calculated for $C_{16}H_{25}N_2O$: 261.1967.

Synthesis of compounds 3a–3g *(general procedure).* A solution of $HClO_4$ in AcOH (5.0 mL, 1.0 N) was added to a solution of **1** (5 mmol) in AcOH (30 mL). The mixture was stirred overnight at room temperature. After complete conversion of **1** (TLC), the solvent was removed under reduced pressure, and EtOH (20 mL) was added to dissolve the residue. The

resulting solution was refluxed until the reaction was complete (TLC), the solvent was removed under reduced pressure, and the residue was purified by crystallization from methanol.

1,3-Diisopropylimidazolium perchlorate (3a). Yield 97%, mp 117–119°C. ^{1}H NMR spectrum, δ, ppm: 1.62 d (12H, J = 6.4 Hz), 4.81 sept (2H, J = 6.4 Hz), 7.87 d (2H, J = 1.2 Hz), 9.17 s (1H). Mass spectrum: m/z 153.1404 $[M - ClO_4]^+$; calculated for $C_9H_{17}N_2$: 153.1392.

1,3-Di-*tert*-butylimidazolium perchlorate (3b). Yield 97%, mp 244–246°C. ^{1}H NMR spectrum, δ, ppm: 1.73 s (18H), 7.99 s (2H), 9.04 s (1H). Mass spectrum: m/z 181.1700 $[M - ClO_4]^+$; calculated for $C_{11}H_{21}N_2$ 181.1699.

1,3-Dicyclohexylimidazolium perchlorate (3c). Yield 98%, mp 174–175°C. ^{1}H NMR spectrum, δ, ppm: 1.25–2.21 m (20H), 4.35 m (2H), 7.47 d (2H, J = 1.6 Hz), 8.99 s (1H).

1,3-Bis(2,6-dimethylphenyl)imidazolium perchlorate (3d). Yield 9%, mp 252–256°C. ^{1}H NMR spectrum, δ, ppm: 2.30 s (12H), 7.37 d (4H, J = 7.6 Hz), 7.51 t (2H, J = 7.6 Hz), 7.77 s (2H), 9.21 s (1H). Mass spectrum: m/z 277.1707 $[M - ClO_4]^+$; calculated for $C_{19}H_{21}N_2$: 277.1705.

1,3-Bis(2,6-diisopropylphenyl)imidazolium perchlorate (3e). Yield 16%, mp 360–365°C. ^{1}H NMR spectrum, δ, ppm: 1.27 d (12H, J = 6.8 Hz), 1.32 d (12H, J = 6.8 Hz), 2.61 sept (4H), 7.55 d (4H, J = 8.0 Hz), 7.71 t (2H, J = 8.0 Hz), 8.42 s (2H), 9.81 s (1H). ^{13}C NMR spectrum. Mass spectrum: m/z 389.2933 $[M - ClO_4]^+$; calculated for $C_{27}H_{37}N_2$: 389.2957.

1,3-Bis[3,5-bis(trifluoromethyl)phenyl]imidazolium perchlorate (3f). Yield 31%, mp 365–367°C. ^{1}H NMR spectrum, δ, ppm: 8.41 s (2H), 8.71 d.d (6H, J = 7.2, 1.6 Hz), 10.53 d.d (1H, J = 1.6, 2.0 Hz). ^{1}Mass spectrum: m/z 493.0589 $[M - ClO_4]^+$; calculated for $C_{19}H_9F_{12}N_2$: 493.0574.

1,3-Bis(2,4,6-trimethylphenyl)imidazolium perchlorate (3g). Yield 10%, mp 230–233°C. ^{1}H NMR spectrum, δ, ppm: 2.11 s (12H), 2.34 s (6H), 7.03 s (4H), 7.60 s (2H), 8.93 s (1H). Mass spectrum: m/z 305.2002 $[M - ClO_4]^+$; calculated for $C_{21}H_{25}N_2$: 305.2018.

Antitumor activity. The cells were cultured in RPMI-1640 and maintained in a Thermo incubator (Waltham, MA) under humidified air containing 5% CO_2. All culture media contained 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution (10 000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl). The cancer cell lines were cultured in minimum essential medium (MEM). Four thousand cells (per well) suspended in MEM were placed into each well of a 96-well plate and incubated for 24 h. The tested compounds were added to the culture medium at the indicated final concentrations, and the cell cultures were incubated for 72 h. Fresh MTT was added to each well to a final concentration of 5 mg/mL and incubated with the cells at 37°C for 4 h. Formazan crystals were dissolved in 100 μL of DMSO for each well, and the absorbance at λ 570 nm was measured using a Synergy 4 multifunctional reading machine. All compounds were tested in triplicate in each cell line. The results expressed as IC_{50} values were the averages of three measurements calculated using GraphPad Prism5 software.

Molecular docking study. Molecular docking of compounds into the 3D CDK2 complex structure (PDB code 1VJP) was carried out using the AutoDock software package as implemented through Auto-Dock Tools (ADT 1.5.2) graphical user interface. Auto Dock Tools (ADT) was used to add polar hydrogens and Gasteiger charges. Ligands were prepared by drawing the 2D structure in CambridgeSoft ChemDraw and converted to PDB format using Chem3D. Ligands were then energy minimized using Discovery Studio 2.1 before using ADT to add polar hydrogens and Gasteiger charges. All bound water and ligands were eliminated from the protein and the polar hydrogen was added. The whole CDK2 complex was defined as a receptor and the site sphere was selected based on the ligand binding location of ATP, then the original ligand molecule was removed and new ligand was placed during the molecular docking procedure. Types of interactions of the docked protein with ligand were analyzed after the end of molecular docking.

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