Electronic Supplementary Information

Carboxyboranylamino ethanol: Unprecedented discovery of boron agent for neutron capture therapy in cancer treatment

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Materials and methods

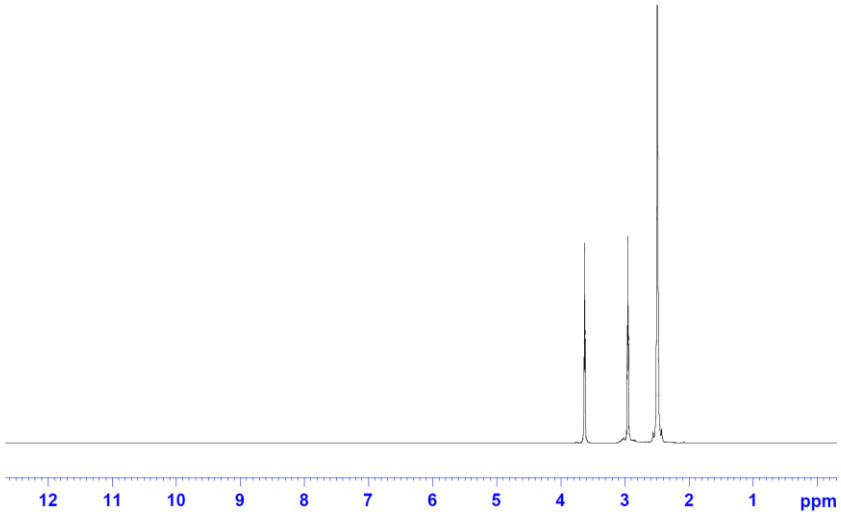
1. Compounds

All reactions were carried out under an argon atmosphere using standard Schlenk-line techniques. Solvents were dried according to the established methods and freshly distilled before use. Trimethylamine carboxyborane was synthesized according to the literature procedure.[1] The BPA and other reagents and organic solvents were purchased from Sigma-Aldrich and were used as received. The carboranyl compound, methyl 2-hydroxyl-5-(1′-*ortho*-carbonylmethyl-1′,2′,3′-triazol-4′-yl)benzonate (**2**) was friendly provided by HEC Pharm Group (Guangdong, China). The FT-IR spectra were measured using an IRTracer-100 SHIMADZU spectrophotometer with KBr pellets. The FT-IR multiplicities are reported as (peak shape, strength): s = singlet, vs = very strong, m = medium, w = weak. Elemental analyses were measured using a EURO EA equipment. High–resolution mass spectra were obtained using Waters Q-TOF Ultima ESI and Agilent 6230 ESI TOF LC/MS spectrometers. The ^{1}H, ^{13}C, and ^{11}B NMR spectra were recorded using a Bruker 200 analyzer at 200, 64.2 and 50.3 MHz, respectively. All NMR spectra were recorded at ambient temperature.

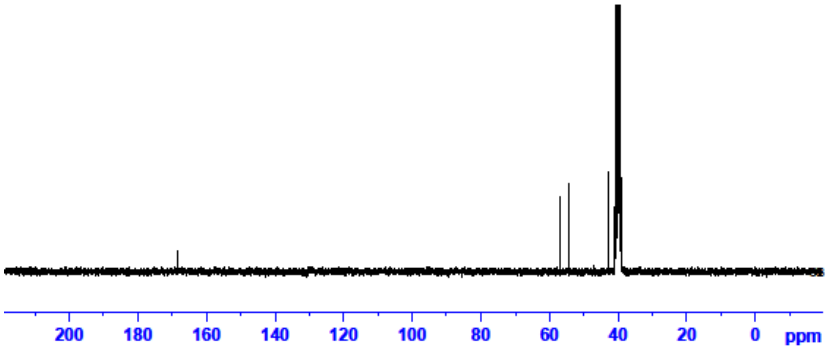
Inductively coupled plasma-optical emission spectroscopy (ICP-OES) measurements were carried out using a VISTA-MPX instrument.

*1.1 Synthesis of **1***

Compound **1** was prepared by an amine exchange reaction in which $Me_3NBH_2CO_2H$ (200 mg, 1.71 mmol) and the 2-(dimethylamino)-ethanol (160 mg, 1.72 mmol) were dissolved in anhydrous tetrahydrofuran (15 mL). The resulting mixture was heated to 70 °C for 5 days using an oil bath. After cooling to room temperature, the solvent in the mixture was evaporated *in vacuo* to produce a pale-yellow sticky residue, as a crude product, which was then purified by recrystallization in tetrahydrofuran and hexane to yield an off-white waxy solid (188 mg) in 75.0 % yield. Elemental Anal: Calcd for $C_5H_{14}BNO_3$: C, 40.86; H, 9.60; N 9.53; B, 32.66. Found: C, 40.60; H, 9.52; N, 9.45; B, 32.48 (determined by ICP-MS measurement). HRMS(ESI): m/z calc. for $C_5H_{14}BNO_3$ $[M+H]^+$: 147.9885 found: 147.9884. ^{1}H NMR (DMSO-d_6, relative to $SiMe_4$; ppm): δ 3.63 (t, 2H, C$\underline{H_2}$-O), 2.93 (t, 2H, C$\underline{H_2}$-N), 2.43 (s, 6H, 2C$\underline{H_3}$). ^{13}C NMR (DMSO-d_6, relative to $SiMe_4$; ppm): δ 166.00 (1C, $\underline{C}O_2H$), 57.26 (1C, $\underline{C}H_2$-N), 56.36 (1C, $\underline{C}H_2$-O), 43.86 (2C, 2$\underline{C}H_3$). ^{11}B NMR (DMSO-d_6, relative to $BF_3{\cdot}OEt_2$; ppm): δ -10.75 (1B, t, $^1J_{BH}$ = 95 Hz). IR (film on KBr, cm^{-1}) 3405 (s, br), 2963 (s, s), 2860 (m, s), 2403 (s, s, ν_{BH}), 1676 (s, s), 1469 (s, s), 1403 (s, m), 1190 (m, s), 1163 (s, s), 1121 (s, s), 1105 (s, s), 1074 (s, s), 992 (m, s), 953 (w, s), 908 (w, s), 866 (m, s), 835 (w, s), 709 (w, s), 523 (w, s).



(a)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ppm

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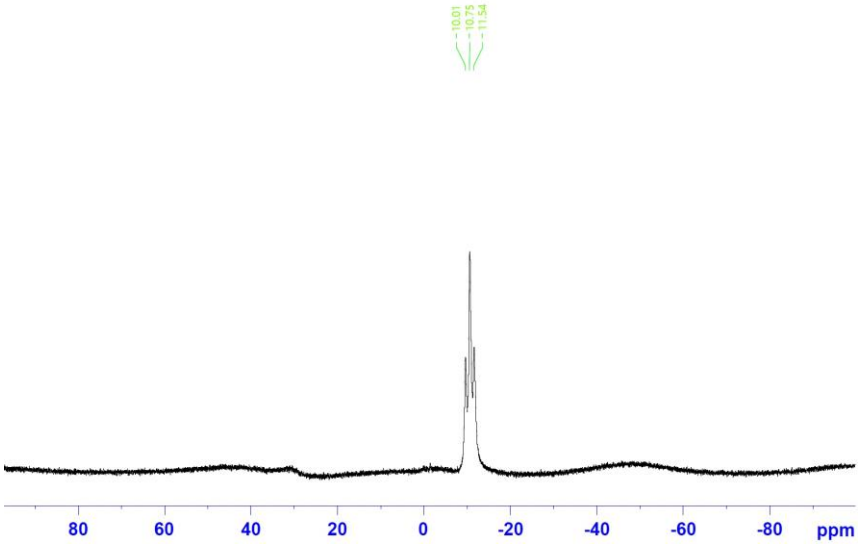
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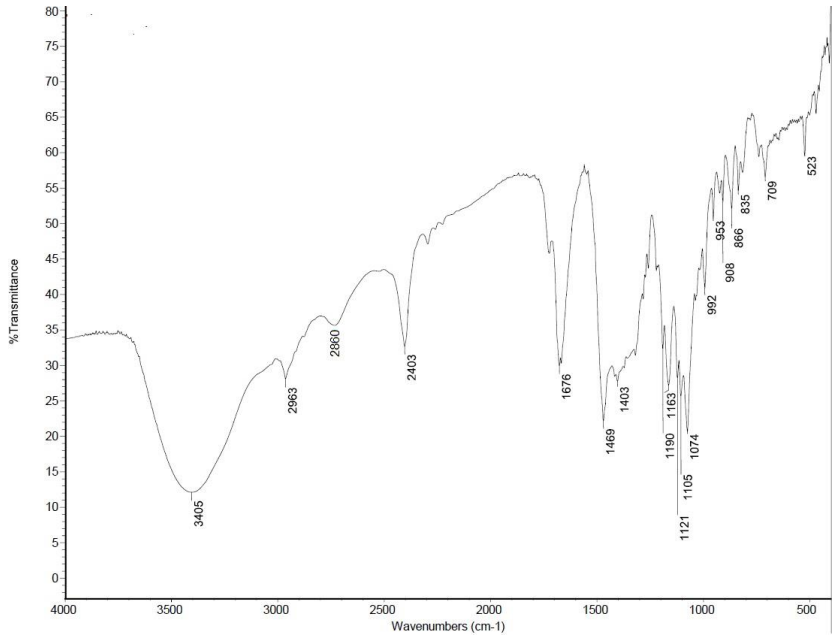
ppm

(c)



(d)

Fig. S-1 ^{1}H NMR (a), ^{13}C NMR (b) and ^{11}B NMR (c) in dmso-d_6 and FT-IR (d) spectra of compound **1**.

2. Preincubation for boron loading into tumor cells

The SCCVII tumor cells were incubated and stabilized in logarithmic growth phase in a 10–cm diameter tissue culture dish. After adding 100 μL of a boron solution described below, the cells were incubated for boron loading into tumor cells for 5 hrs. The detailed conditions of the preincubation and the corresponding ^{10}B uploading amounts of compounds **1** and **2** were as following:: sample concentration, 20 mg/mL; microscopic observation of tumor cells after pre-incubation, clear & not toxic; adding amount of MEM, 100 μL/10mL; pre-incubation ^{10}B concentration, 0.37 ppm for compound **1** and 1.2 ppm for compound **2**; ^{10}B concentration determined by prompt γ-ray assay, 36.7 ppm for compound **1** and 116.4 ppm for compound **2**.

3. Cytotoxicity: IC_{50}

The IC_{50} (moles/liter), i.e., the concentration which inhibits the growth of SCCVII tumor cells by 50% after 3 days of continuous exposure to the compounds in the concentration gradient manners, was determined. Suspensions of 10^5 SCCVII cells/200μL MEM containing 10% FCS (fetal cow serum) were incubated for 3 days in 96-well microplate with various concentrations of **1** and **2**. After 3-days of exposure, the cells were washed with PBS and fixed with 70% ethanol. The SCCVII cells were stained with 5 % Giemsa solution for 15 minutes. After removing the solution, the cells were dried in air and Giemsa-stained cells were uniformed by adding 100μL alcohol. The optical absorbance at 595 nm was measured by optical densitometer. The IC_{50} was determined as the dose producing a 50% density reduction in the optical absorbance. The results are presented in Fig. 1(B).

For IC_{50} analysis of the uppsala 87 malignant glioma (U87MG) cell lines, malignant melanoma A3750 cell lines, rat cortical neuron cell lines and rat fibroblast like synoviocytes (FLS) cell lines, the standard MTT method was used to analyze IC_{50} of compound **1** and BPA. The IC_{50} determinations were performed in the presence of serial dilutions of inhibitor (1000μM to 3.906μM, 1% DMSO, final) in complete reaction buffer and incubated for 96 hours. The IC50 values of compound **1** and BPA are greater than 1000μM for all the examined cell lines.

4. Boron uptake assay

The $3.0x10^8$ rat FLS cells or $2.5x10^7$ U87MG cells were seeded and cultured at room temperature (37 ℃, 5%CO) for 24hrs. This culture fluid was removed therefrom by suction, culture fluids containing 1.5 mM of each boron agent (**1** and BPA in the boron concentrations of 3.0Mm and1.5mM, respectively) were added and continued culturing for 24hrs under same conditions. These culture fluids were removed by suction and the cells were washed three times with PBS and treated with trypsin to recover the cells. The number of cells recovered was counted, and HNO_3 (2N, 1.5 ml) was added and the resulting mixture was heated at 80 ℃ for 12hrs. After filtering with a membrane filter, the boron concentration was determined by ICP-OES. The results are as follows: **1**, 1.27 (U87MG) and 0.54 (FLS) μg boron/10^7 cells; BPA, 0.49 (U87MG) and 0.55 (FLS) μg boron/10^7 cells.

5. Survival study: colony formation assay

A cell suspension of SCCVII in the logarithmic growth phase was prepared in Eagle's MEM containing 10% heat-inactivated fetal bovine serum and 2mM l-glutamine. The cells were incubated in the culture dishes (100-mm tissue culture dishes; Corning Glass Works, Corning, NY) at a sub confluent concentration of 5 x 10^4 cells /10ml/dish, overnight at 36℃ in a 5% carbon dioxide atmosphere. A 100 mL solution of 6.0 mg of **1** and/or **2**/mL DMSO was added into the dish for boron loading into tumor cells and incubated for 5 hrs. The ^{10}B concentration (0.37 ppm for **1** and 1.2 ppm ^{10}B for **2**) in the medium was reconfirmed by PGS (prompt γ spectroscopy). After boron loading, the cells were trypsinized and washed three times by PBS, and $5x10^3$ cells/mL MEM(FCS+) were irradiated with thermal neutrons in column-shaped Teflon tubes (1x3 cm). The cells did not adhere to the tubes, and no secondary radiation was caused by bombardment with the thermal neutrons. The irradiation times were 0, 15, 30 and 45 minutes, while the thermal neutrons flux was $1.8x10^9$ n/cm^2. The thermal neutron fluence was determined by averaging two gold foils symmetrically attached to the surface of the Teflon tube along the direction of the incidence of the thermal neutrons.[2,3] The neutron absorbed dose (Gy) was calculated using the flux-to-dose conversion factor.[4] The chemical composition of the tumors was assumed to be 10.7% hydrogen, 12.1% carbon, 2% nitrogen, 71.4% oxgen and 3.8% others.[5] The γ-ray dose rate, including secondary γ-rays, was $1.0x10^{-2}$ Gy·min^{-1}, according to a thermoluminescence dosimeter attached to the surface of a Teflon tube

containing 1 mL MEM. After thermal neutron exposure, 300 or 900 cells were placed in three Corning 60-mm tissue culture dished containing 6.0 mL MEM to examine colony formation. Ten days later, the colonies were fixed with 70% ethanol and stained with 5% Giemsa solution for quantitative visualization by the naked eye. Values are represented as means ±SE. Three replications of this *in vitro* BNCT experiments were performed. The *in vitro* cell-killing effects are shown in Fig. 1 (C).

6. Tissue distribution assay.

The tissue distributions of compound **1** in dimethyl sulfoxide solvent were measured using six-week old female nod-scid mice. The mice were housed and treated humanely under standard conditions. U87MG tumor cells, a mammary carcinoma, were then transplanted into the right flank of the young female nod-scid mice of ~20g body weight four weeks before testing. The dosage was 120 mg/Kg and the compound concentration was 30mg/ml in a solution of DMSO (10% v/v)/RH40 (10% v/v)/sterilized water (80% v/v) for injection. The solution was slowly injected into the tail vein of the mice. For comparison, five tissues, tumor, blood, brain liver, and skin samples were collected and analyzed with ICP-OES. The mice were anesthetized (diethyl ether) and bled into heparinized syringes via cardiac puncture before surgery to collect blood. The collected blood was then placed into tared cryogenic tubes and kept frozen at -60 ℃. The mice were later sacrificed via cervical dislocation while anesthetized. The tumor and organs samples were collected, placed in tared cryogenic tubes, and kept frozen at -60 ℃ before being subjected to analysis with ICP-OES. The results are shown in Fig. S-2. Each data point represents the average of three mice.

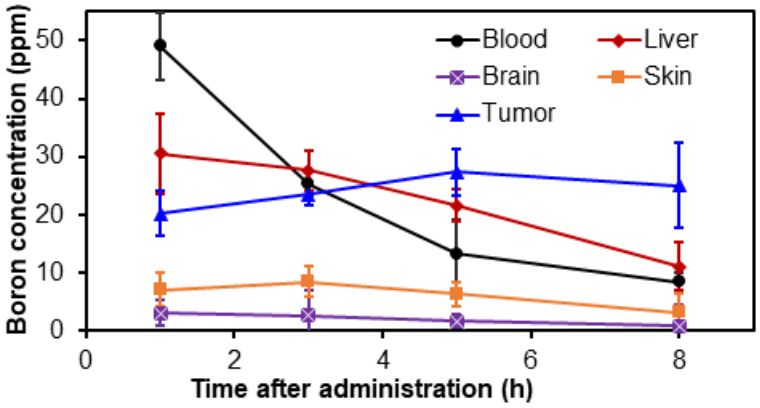


Fig. S-2 Tissue distribution of compound **1**.

7. Statistical analysis

Statistical analysis and drawing graphs were carried out via Prism 9 for macOS version 9.02, GraphPad Software, LLC. The *in vitro* BNCT was carried out in triplicates. The values are the mean ±SEM from three independent experiments. The significance of the differences in survival rates was assessed by Student's *t* test. The

statistical analyses were performed using Prism 3.0 (GraphPad Software Inc., CA, USA).

8. Live subject statement

All experiments were performed in compliance with the relevant laws and institutional guidelines, and the Animal Ethical Committee of HEC Pharma Co., Ltd. (Dongguang, Guangdong, China) have approved the experiments.

9. Molecular docking

9.1 Molecular docking calculation

The molecular operating environment (MOE) software (Chemical Computing Group 2016) and protein-small molecular docking study Patch dock open-source server (http://bioinfo3d.cs.tau.ac.il/PatchDock/),[6-9] which can predict favorable protein-ligand complex structures with high accuracy have been used to calculate the molecular interactions between ligand (small molecule) and receptor (protein). The three-dimensional (3D) crystal structures of the epidermal growth factor receptor (EGFR), 3W32, 3W33, 3BEL, 3BUO, 4I22, 4I24 and 4R3R proteins were retrieved from the Protein Data Bank (PDB) (*www.rcsb.org*). Solvent impurities and co-crystallized ligand molecule and all water molecules were manually removed by Pymol 2.4 Software package, and then saved the proteins as .pdb files after adding polar hydrogen atoms to the proteins. Both PDB files were uploaded into the Patch dock server for protein-small molecular docking simulation. Docking was performed with complex type configuration settings. The PatchDock server follows a geometry-based molecular docking algorithm to find the docking transformations with good molecular shape complementarity. The PatchDock algorithm separates the Connolly dot surface representation of the molecules into concave, convex and flat patches. These divided complementary patches are matched in order to generate candidate transformations and evaluated by geometric fit and atomic desolvation energy scoring functions. The results show binding sites for **1**, **2** and BPA. The association details of each binding site are presented using the "ligand interactions" module of MOE. The calculated results of geometric shape complementarity score (GSCS) are listed in Table S-1. The docking models of BPA, **1** and **2** with EGFR 3W32 proteins are shown in Fig. 2. The active site pockets of 3W32 protein bound with BPA, **1** and **2** were generated by Pymol 2.4 and Chemdraw 10.0 software packages as shown in Fig. S-1.

Table S–1. The calculation of geometric shape complementarity score (GSCS).

EGFR / Compd	3W32	3W33	3BEL	3BUO	4I22	4I24	4R3R

1	5092	4562	4904	4438	4988	4794	4854
2	3664	3410	3602	3400	3342	3162	3468
BPA	2692	2692	2928	2840	2572	2590	2624

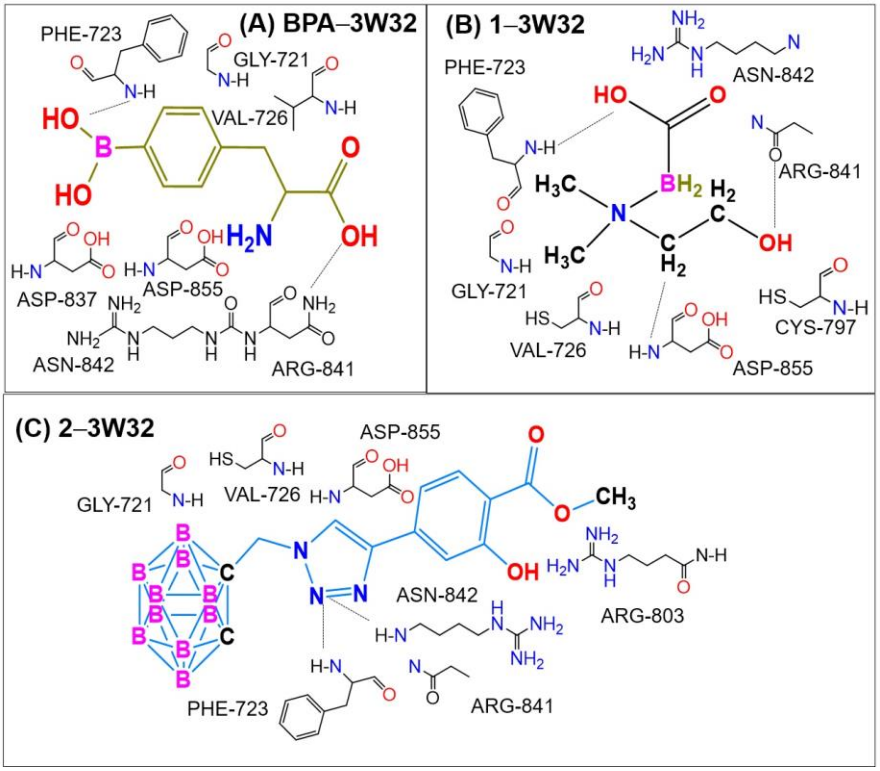


Fig. S-3 Interactions (hydrogen bonds) of amino acid residues of EGFR protein 3W32 with BPA (A), **1** (B) and **2** (C). The Figure was generated using Chemdraw 10.0 and Pymol 2.4 software.

Table S–2. The calculation of geometric shape complementarity score (GSCS).

CMYC / Compd	**1A93**	**2A93**	**1EE4**	**5I4Z**	**5I50**	**6E24**	**6C4U**
1	4184	4230	4296	4202	4842	4696	5096
2	2778	2758	2814	2804	3298	3032	3396
BPA	2048	1928	2518	1900	2470	2332	2456
CMYC / Compd	**1NKP**	**2OR9**	**4Y7R**	**6G6J**	**6G6L**	**6G6K**	**6E16**
1	5182	4728	4152	4764	4562	5222	4684
2	3410	3086	3052	2872	3010	3236	3052
BPA	2552	2262	2158	2232	2298	2566	2250

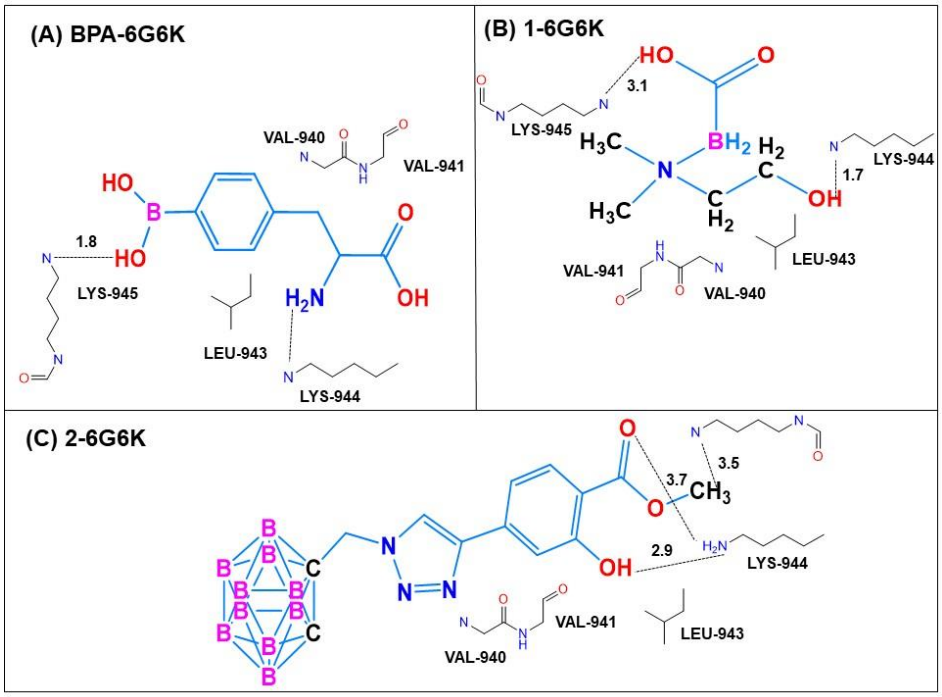


Fig. S-4 Interactions (hydrogen bonds) of amino acid residues of CMYC protein 6G6K with BPA (A), **1** (B) and **2** (C). The Figure was generated using Chemdraw 10.0 and Pymol 2.4 software.

The binding energy was calculated using a Molecular Operating Environment (MOE) software (MOE 2016.08 software package, Chemical Computing Group ULC, Montreal, Quebec, Canada). The software establishes a good correlation between the binding strength and various physico-chemical parameters. The coefficients were fitted from approximately 400 X-ray crystal structures of protein-ligand complexes with available experimental pKi data. Atoms are categorized into about a dozen atom types for the assignment of the ci coefficients. The triple integrals are approximated using Generalized Born integral formulas. The proteins were downloaded from online RCSB Protein Data Bank (PDB). Energy minimization was performed using defaults parameters in MOE by applying the AMBER10 force field.[10] The conformational searching of the compounds **1**, **2** and BPA was conducted by the LowModeMD method. The docking function of MOE can give the correct conformation of the compounds **1**, **2** and BPA to obtain minimum energy structure.

9.2 Molecular docking results of 1 (MUST1) with various EGFR proteins.

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3w32.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	5092	611.60	-279.85	-1.22 1.36 -0.97 27.99 33.45 -4.69

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3w33.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4562	604.30	-245.10	2.20 0.28 2.58 18.34 33.50 12.89

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3bel.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4904	595.90	-124.73	2.13 -0.14 -2.37 17.65 35.12 91.09

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3buo.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4438	541.50	-52.68	0.92 -0.45 -1.52 -5.07 5.60 10.59

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4i22.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4988	567.40	-83.64	1.73 -0.66 -0.26 14.42 -14.48 17.73

Molecular Docking Algorithm Based on Shape Complementarity Principles

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4i24.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4794	523.80	-146.79	1.48 -0.33 -1.48 -14.09 34.32 2.21

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4r3r.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4854	584.70	-153.80	2.08 1.23 0.42 -65.58 7.05 -31.77

9.3 Molecular docking results of 2 (HEC1) with various EGFR proteins.

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3w32.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3664	390.30	-153.08	0.97 0.70 0.40 17.77 34.32 16.24

Molecular Docking Algorithm Based on Shape Complementarity Principles

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3w33.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3410	422.00	-140.70	1.25 -0.35 -0.90 16.71 32.05 9.77

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3bel.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3602	419.60	-132.33	2.50 -0.34 -1.88 15.94 35.98 93.58

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3buo.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3400	401.50	-91.69	1.00 -0.44 -2.30 -3.65 3.87 11.54

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4i22.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3342	397.40	-151.99	-1.59 -0.07 -0.23 11.79 -14.94 11.79

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4i24.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3162	336.20	-12.14	2.52 -0.26 -0.27 -1.81 48.71 -9.02

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4r3r.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3468	390.40	-98.78	-0.44 1.39 -0.16 -63.72 8.66 -32.63

9.4 Molecular docking results of BPA with various EGFR proteins.

Molecular Docking Algorithm Based on Shape Complementarity Principles

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3w33.pdb	BPA1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2692	294.00	-129.49	-2.52 0.55 -0.13 16.53 31.11 7.81

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3w32.pdb	BPA1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2692	298.70	-104.21	2.90 -1.18 -2.59 26.31 34.38 -3.29

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3bel.pdb	BPA1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2928	324.50	-94.98	-1.79 -0.42 1.48 15.42 36.03 94.76

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
3buo.pdb	BPA1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2840	298.60	-86.23	2.38 -0.37 2.01 -1.64 3.90 12.78

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4i22.pdb	BPA1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2572	297.10	-67.02	-0.13 0.77 2.87 11.18 0.15 -3.50

Molecular Docking Algorithm Based on Shape Complementarity Principles

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4i24.pdb	BPA1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2590	282.50	-1.40	-0.56 -0.18 -1.36 -2.65 47.86 -7.88

Molecular Docking Algorithm Based on Shape Complementarity Principles

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4r3r.pdb	BPA1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2624	286.50	-69.23	1.65 -0.50 -2.44 -62.31 9.67 -33.72

9.5 Molecular docking results of 1 (MUST1) with various c-myc proteins.

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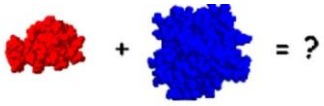
Molecular Docking Algorithm Based on Shape Complementarity Principles



Molecular Docking Algorithm Based on Shape Complementarity Principles

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
1a93.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4184	515.10	-45.20	2.77 -0.07 2.78 47.00 42.77 2.01

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
2a93.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4230	514.90	-86.69	2.47 -0.69 -1.31 3.64 -14.39 3.77

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
1ee4.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4296	510.00	-45.93	0.20 0.87 2.23 0.29 -1.10 41.77

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
5i4z.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4202	574.10	-115.94	1.78 0.97 -1.65 51.00 23.80 7.48

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
5i50.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4842	578.00	-129.26	1.98 -1.34 -3.10 -0.85 -8.42 -29.15

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6e24.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4696	548.00	-125.77	-1.96 -0.78 1.35 177.79 1.41 14.48

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6c4u.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	5090	602.90	-132.21	2.44 -0.55 -2.26 34.61 -26.71 -58.71

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
1nkp.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	5182	638.10	-74.28	-2.53 -0.19 1.19 32.22 27.73 92.80

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
2or9.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4728	534.00	-165.26	-0.02 -0.55 -0.67 62.69 101.53 -28.53

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4y7r.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4152	497.10	-72.80	-0.26 0.23 2.26 10.04 1.92 19.31

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6g6j.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4764	579.80	-92.00	-2.88 0.83 -2.98 49.85 89.47 195.86

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6g6l.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4562	554.40	-80.45	1.85 0.72 -1.94 -98.42 29.03 -48.05

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6g6k.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	5222	638.00	-140.07	-1.71 -0.34 2.96 -55.03 -41.21 -73.26

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6e16.pdb	MUST1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	4684	528.10	-55.55	-2.71 0.57 -2.93 106.75 71.34 -13.81

9.6 Molecular docking results of 2 (HEC1) with various c-myc proteins.

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
1a93.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2778	353.30	-79.94	-1.68 -0.45 1.08 44.53 6.92 -1.80

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
2a93.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2758	332.30	-49.34	-1.98 -0.26 1.39 2.62 -15.33 3.64

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
1ee4.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3080	369.80	-71.68	2.30 0.31 2.66 8.94 46.99 103.51

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
5i4z.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2804	352.80	-91.20	-1.32 0.36 1.15 50.89 28.78 8.88

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
5i50.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3296	388.90	-68.17	0.17 0.43 -2.37 -1.42 -10.09 -30.64

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6e24.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3032	342.20	-107.17	0.75 -0.84 1.03 178.57 -0.93 12.23

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6c4u.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3396	387.60	-32.72	-2.45 -0.11 0.49 63.94 -6.64 -22.41

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
1nkp.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3410	377.10	59.70	-0.62 -1.20 -0.34 54.69 54.62 48.69

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
2or9.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3086	332.00	-91.72	1.29 -0.30 -0.48 67.02 75.14 -7.12

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4y7r.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3052	321.70	-134.10	-2.32 0.88 0.71 14.43 27.72 16.77

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6g6j.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2872	325.40	-52.93	1.25 -0.91 1.38 24.74 16.15 172.18

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6g6l.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3010	356.10	8.02	0.17 1.22 1.04 -78.29 -11.22 -57.36

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6g6k.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3236	406.10	-66.44	1.27 0.16 2.74 -57.63 -41.98 -67.64

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6e16.pdb	HEC1.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	3052	345.10	-79.17	1.39 -0.44 -1.99 104.76 72.83 -14.38

9.7 Molecular docking results of BPA with various c-myc proteins.

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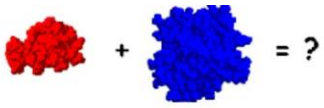

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
1a93.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2048	220.20	1.71	-2.66 -0.27 -1.50 47.53 38.28 -0.36
2	2008	261.70	-61.43	-1.17 0.71 -2.28 43.24 7.21 0.47

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
2a93.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	1928	207.70	-41.31	-0.69 -0.06 -1.40 -5.41 -16.17 -4.31

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
1ee4.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2518	304.40	-35.18	-2.36 -0.80 -2.59 8.77 43.77 100.60

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
5i4z.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	1900	208.80	-56.50	2.04 -1.42 1.05 23.58 32.47 11.58

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
5i50.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2470	284.20	-21.12	-0.20 0.56 -3.11 -4.42 -8.24 -27.39

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Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6e24.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2332	251.00	-54.59	-3.06 -1.07 0.34 179.06 -1.17 11.88

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6c4u.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2456	268.50	-104.44	3.13 -0.14 0.45 38.30 -24.32 -61.07

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
1nkp.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2552	271.10	18.31	1.09 -0.40 2.09 54.11 54.76 49.19

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
2or9.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2262	278.40	-74.31	0.68 1.46 1.74 -7.54 19.66 27.57

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
4y7r.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2158	237.40	-54.05	-2.40 0.64 -1.82 3.36 34.67 21.74

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6g6j.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2232	283.20	3.03	2.98 -0.28 -1.73 47.41 90.76 205.24

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6g6l.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2298	288.60	22.48	1.76 0.31 2.14 -107.16 4.13 -15.93

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6g6k.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2566	316.80	-59.63	1.61 0.04 0.17 -56.83 -42.21 -68.42

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail
6e16.pdb	BPA.pdb	drug	1.5	1909853wct30001@student.must.edu.mo

Solution No	Score	Area	ACE	Transformation
1	2250	251.10	-59.73	0.61 1.11 -0.89 104.97 73.94 -13.93

10. Analysis data of 2.

Product name: **methyl 2-hydroxyl-5-(1′-*ortho*-carbonylmethyl-1′,2′,3′-triazol-4′-yl)-benzonate.**
Product information: Compound no: HEC156312, off-white solid.
Analysis: ^{1}H NMR (400MHz, $CDCl_3$) δ 10.85(s, 1H), 7.92(d, 1H), 7.87(s, 1H), 7.41-7.43(m, 2H), 5.07(s, 2H), 3.97(s, 3H), 3.90(s, 1H), 1.86-3.00(m, 10H). LC-MS(ES-AP): $[M+H]^+$ = 377.30. HRMS: $C_{13}H_{21}B_{10}N_3O_3$, m/z: 377.2643. HPLC purity: 98.64%.

References

1 B. E. Spielvogel, F. U. Ahmed and A. T. McPhail, Compounds of pharmacological

interest, in *Inorganic Syntheses* (H. R. Allcock, Editor), Singapore, John Wiley & Sons, 1989, **25**, 79.
2 D. W. Rogers, *Health. Phys.*, 1984, **46**, 891.
3 R. L. Maughan, P. J. Chuba, A. T. Porter, E. Ben-Josef and D. R. Lucas, *Med. Phys.*, 1997, **24**, 1241.
4 R. S. Caswell, J. J. Coyne and M. L. Randolph, *Radiat. Res.*, 1980, **83**, 217.
5 W. S. Snyder, M. J. Cook, E. S. Nasset, L. R. Karhausen, G. P. Howells and I. Tipton, Cross and elemental content of reference man. In *Report of the task Group on Reference Man* (W. S. Snyder, Ed.). pp. 273–324. Pergamon Press, Oxford, 1975.
6 P. T. Lang, S. R. Brozell, S. Mukherjee, E. F. Pettersen, E. C. Meng and V. Thomas, et al., *RNA (New York, N.Y.)*, 2009, **15,** 1219.
7 D. Schneidman-Duhovny, Y. Inbar, R. Nussinov, H. J. Wolfson, *Nucl. Acid Res.*, 2005, **33**, W363.
8 S. Salentin, S. Schreiber, V. J. Haupt, M. F. Adasme, M. Schroeder, *Nucl. Acids Res.*, 2015, **43(W1)**, W443.
9 W. L. De Lano, The PyMOL Molecular Graphics System. San Carlos, CA, USA: De Lano Scientific, 2004.
10 R. S. S. Murali, R. S. S. Siddhardha, D. R. Babu, S. Venketesh, R. Basavaraju and G. N. Rao, *Spectrochim. Acta Part A: Mol. Biomol. Spectr.*, 2017, **180,** 217.