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Syntheses, cytotoxic activity evaluation and HQSAR study of 1,2,3-triazole-linked isosteviol derivatives as potential anticancer agents

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Abstract: A series of novel 1,2,3-triazole-linked isosteviol derivatives were designed and synthesized *via* Huisgen-click reaction. Their cytotoxicities *in vitro* against HCT-116 and JEKO-1 cells were screened. The preliminary bioassays indicated that most of the title compounds exhibited noteworthy cytotoxic activities. Particularly, the compound **10b** revealed the most potent inhibitory activities against HCT-116 cells with IC_{50} value of 2.987 ± 0.098 μM, which was better than that (3.906 ± 0.261 μM) of positive control cisplatin. On the basis of these bioactivity data, hologram quantitative structure–activity relationship (HQSAR) was performed, and a statistically reliable model with good predictive power (r^2=0.848, q^2=0.544 and R^2_{pred}= 0.982) was achieved. Additionally, the contribution maps derived from the optimal model explained the individual atomic contributions to the activity for each molecule.

Key words: Isosteviol; Synthesis; Cytotoxicity; Click chemistry; HQSAR.

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Cancer is becoming the second leading cause of death after cardiovascular disorders throughout the world. For the treatment of cancer, many chemotherapeutic agents, such as 5-fluorouracil, cisplatin, doxorubicin and cetuximab, have been developed over the past few decades.[1-5] However, the resistance of cancer cells to cytotoxic drugs can lead to treatment failure. Therefore, it is utmost significant and urgent to develop remarkable chemotherapeutic agents having a limited

toxicity profile.

Isosteviol (*ent*-16-ketobeyeran-19-oic acid **1**), a tetracyclic diterpenoid with an *ent*-beyerane skeleton that possesses multifarious bioactivities, can be readily obtained as a metabolite of stevioside isolated from the leaves of the natural *stevia* plant.[6,7] In recent years, isosteviol and a number of its derivatives have been shown to exhibit a wide range of biological activities, such as anticancer, antihypertension, antihypotension, antihyperglycaemic, cardio and neuro protective effects.[8-17] Some special group hybridized isosteviol derivatives have shown the promising inhibitory activity against cancer cells. For example, isosteviol-fused pyrazole derivatives **A** (Fig. **1**), obtained by means of functional interconversions in ring D of isosteviol in our laboratory, exhibited growth inhibitory effects comparable to cisplatin *in vitro* against gastric (SGC-7901), lung (A549), lympha (Raji) as well as cervical (Hela) carcinoma cells,[18] which prompted us to further investigate new heterocycle-linked isosteviol derivatives to develop novel stronger anticancer agents for therapeutic use.

1,2,3-Triazoles, highly nitrogen-rich heterocyclic compounds, have been regarded as an interesting unit in terms of biological activity and some of them have shown significant anticancer activity in many of the human cell lines such as colon, lung, prostate, breast cancers and so on.[19-25] Banday and co-workers[26] reported that a series of 1,2,3-triazolyl derivatives of pregnenolone **B** (Fig. **1**) showed significant cytotoxicities against colon (HCT-15, 502713), prostrate (DU-145, PC-3), liver (HEP-2) and lung (A-549) cancer cell lines with IC_{50} values 0.03–8.63 μM. In addition, Khaybullin et al.[27] revealed that the inhibitory activity against breast (MDA-231), lung (A549), pancreas (ASPC-1), prostate (PC-3), colon (HCT-116) and cervical (HeLa) cancer cells were greatly improved as 1,2,3-triazole ring was introduced onto 19-carboxyl group of isosteviol **C** (Fig. **1**). Meanwhile, these reports explicitly pointed out that substituted 1,2,3-triazole rings played an important role in inhibiting the growth of cancer cells, thus allowing new possibilities for quantitative structure-activity relationship studies associated with structure-based good bioactivity molecule design strategies. However, few reports have been focused on the activity relationship of 1,2,3-triazole-linked isosteviol derivatives. In view of these results, the objective of the current work is to synthesize novel 1,2,3-triazole-linked isosteviol derivatives through Huisgen-click reaction, and to investigate *in vitro* their cytotoxicities against colon (HCT-116), mantle cell lymphoma (JEKO-1) cancer cell lines. On the basis of the novel molecule structures

and their cytotoxicities, the robust and predictive model will be developed by the HQSAR technique, and be further taken advantage of designing new anticancer molecules with improved potency.

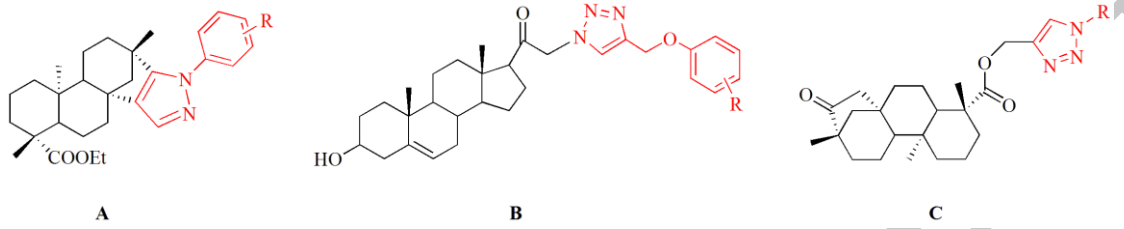
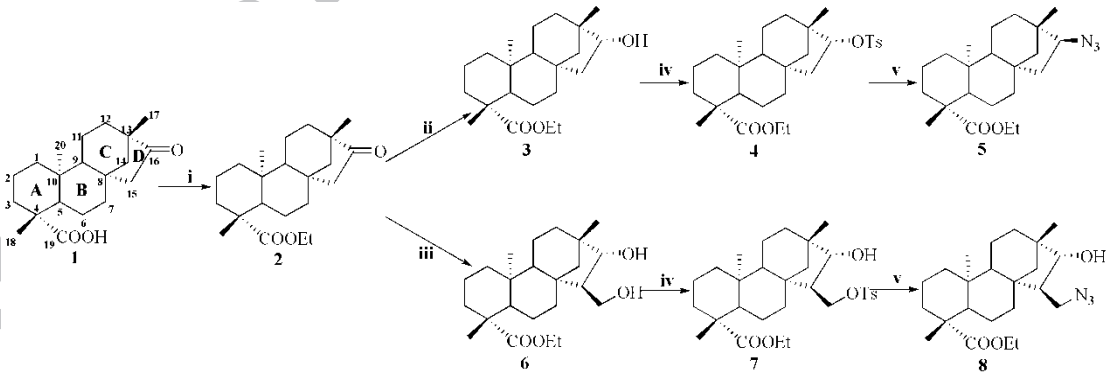


Fig. 1. Chemical structures of compounds **A**-**C**

In an effort to discover new target molecules with special anticancer potency, some structural modifications were made at the C15 and C16-positions of isosteviol by preparing different substituted 1,2,3-triazole derivatives. It is well known that Huisgen-click reaction of terminal alkynes and organic azides to give five-membered 1,2,3-triazoles, a class of 'click' chemistry, has emerged as a powerful linking reaction and found widespread applications ranging from combinatorial drug research and material science to bioconjugate chemistry. Therefore, our initial efforts were focused on the synthesis of intermediates **5** and **8,** with azido group at different places, to be suitable for Huisgen-click reaction. The synthetic routes were outlined in Scheme **1**.

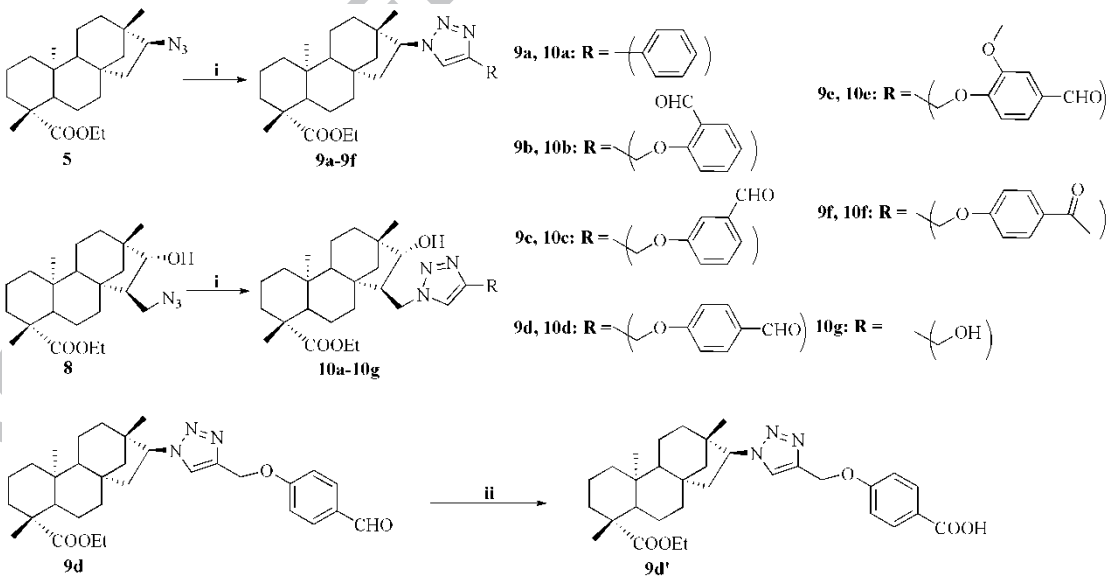


Scheme 1. *Reagents and conditions:* (i) CH_3CH_2Br, DMSO, KOH, rt, 6h, 97%; (ii) $NaBH_4$, C_2H_5OH, rt, 6h, 98%; (iii) HCHO, C_2H_5ONa, C_2H_5OH, 55°C, 8 h, 85%; (iv) TsCl, pyridine, rt, 36h; (v) NaN_3, DMF, 85°C, 8h.

Isosteviol **1**, obtained through acidic hydrolysis of stevioside, was treated with CH_3CH_2Br and KOH in DMSO to yield the corresponding isosteviol ethyl ester **2**. The 16-α-hydroxyl isosteviol derivative **3** was prepared as a white powder by reduction of compound **2** with $NaBH_4$ in

anhydrous C_2H_5OH, whereas the 15-β-hydroxymethyl-16-α-hydroxyl isosteviol derivative **6** was stereo-selectively synthesized *via* a "one-pot" two-steps Tollens' reaction. The absolute configurations of the compounds **3** and **6** were determined according to the literature.[28] Treatment of compound **3** or **6** with 1.1 equiv. of 4-Methylphenylsulfonyl chloride in pyridine readily afforded the corresponding product **4** or **7** in good yield. Reaction of compound **7** with NaN_3 in dry DMF furnished the desired azide product **8** in high yield with excellent purity. However, at the same reaction conditions, compound **5** was obtained with much lower yield, due to the steric effects of the neighboring groups adjacent to C16. The absolute configuration of compound **5** was indirectly confirmed by X-ray crystallographic analysis of its 1,2,3-triazole derivative **9a**, demonstrating a β orientation of the azido group at C-16.

Although procedures for Cu (0) and Cu (I)-catalyzed combination of organic azides and terminal alkynes have been well developed, they were not suitable for our desired hybrids. After investigating several Cu (II)-ligands catalyst, we found that 1,2,3-triazole-linked isosteviol derivatives were exclusively obtained with no side products by employing copper (II) acetate and sodium ascorbate as the catalytic system.



Scheme 2. *Reagents and conditions:* (i) the relative alkynes, sodium ascorbate, $Cu(OAc)_2$, *tert*-butanol/H_2O, rt; (ii) Jones reagent, CH_3COCH_3, rt, 1h, 97%.

Huisgen-click reaction of the azides (**5** or **8**) and appropriate substituted terminal alkynes, such as alkyl-, phenyl-substituted alkynes as well as alkynes containing *ortho*-, *meta*-, *para*-aldehyde, *para*-ketone and 2-methoxyl-4-aldehyde groups on the aromatic ring, readily took place at room

temperature in the presence of copper (II) acetate and sodium ascorbate as catalyst to afford the corresponding 1,2,3-triazole derivatives **9a-9f** in yields of 88-94% and **10a-10g** in yields of 87-99%, respectively (Scheme **2**). In order to further investigate the effect of stronger electron-withdrawing substituent on the aromatic ring on cytotoxic activities, the aldehyde group in compound **9d** was converted to the carboxyl group *via* Jones reagent oxidation in acetone in order to compare the effect of different groups at the benzene ring. Structures of these novel compounds were elucidated on the basis of spectroscopic data. The IR spectrum of compound **9a,** as a representative example of the synthesized compounds, showed moderate absorbance peak at 3145 cm^{-1} due to the formation of 1,2,3-triazole ring leading to stretching vibration of associated C=C–H. The ^{1}H NMR spectrum of compound **9a** displayed the characteristic 1,2,3-triazole ring proton at δ 7.72 ppm in addition to the C16-H signal at δ 4.63 ppm. The ^{13}C NMR spectrum of compound **9a** revealed two carbons of the 1,2,3-triazole ring appearing at δ 147.10 and 130.88 ppm, respectively. In accord with the molecular formula $C_{30}H_{42}N_3O_2$, an $[M + H]^+$ peak at *m/z* 476.3277 in the HRMS was observed. Furthermore, a conclusive evidence for the assigned structure was provided by its X-ray crystallographic analysis (Fig. **2**) [29].

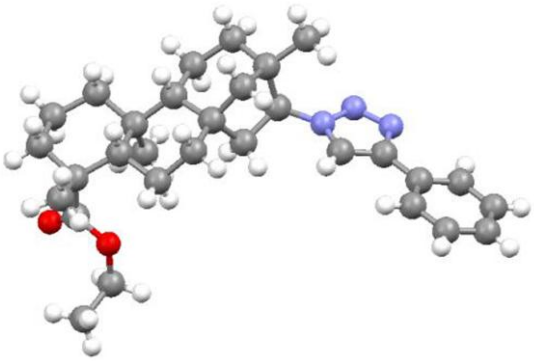
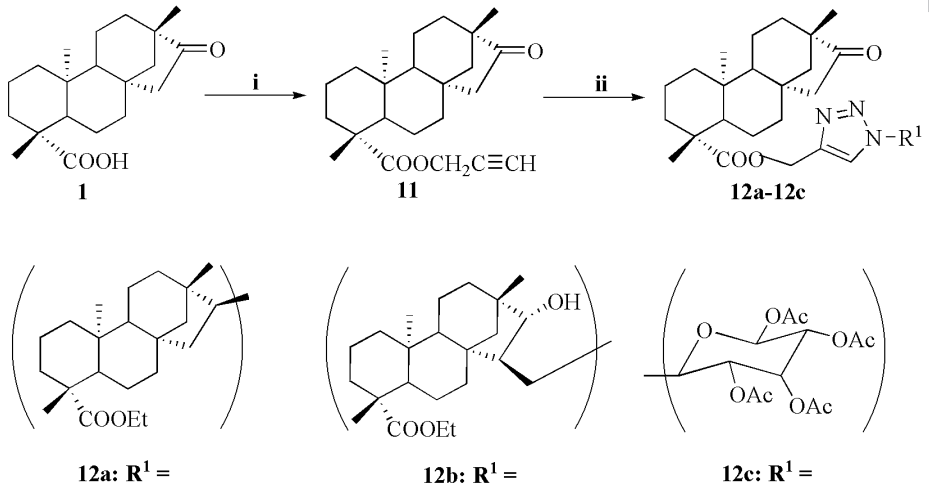


Fig. 2. X-ray structure of compound **9a**

Since the structural modifications of 19-carboxyl group could result in the bioactive improvement in previous report, [8] we intended to introduce the 1,2,3-triazole fragment to the C19-position of isosteviol in order to construct a novel family of bioactive molecules for the discovery of anticancer agent, and simultaneously to provide diverse molecule structures convenient for the subsequent HQSAR study. Therefore, novel 1,2,3-triazole-linked C19-position of isosteviol derivatives **12a-12c** were designed and synthesized by Huisgen-click reaction. The synthetic approaches employed to prepare the target derivatives were outlined in Scheme **3**. Treatment of

compound **1** with propargyl bromide under alkaline conditions in DMSO offered the corresponding alkyne **11**, which further reacted with the substituted azides in the presence of copper (II) acetate and sodium ascorbate as catalyst to give the target products **12a-12c** in good yield. In the IR, NMR and HRMS spectra of **12c**, additional signals were distinctly observed at ν 3142 cm^{-1}, δ_H 7.82 ppm, δ_C 143.58, 122.16 ppm and *m/z* 738.3216 (theoretical value: 738.3214) indicating the formation of 1,2,3-triazole ring.



1 **11** **12a-12c**

12a: R^1 = **12b: R^1 =** **12c: R^1 =**

Scheme 3. *Reagents and conditions:* (i) 3-bromo-1-propyne, DMSO, KOH, rt, 1h, 84%; (ii) the relative azides, sodium ascorbate, $Cu(OAc)_2$, *tert*-butanol/H_2O, rt.

These newly synthesized isosteviol derivatives were further utilized to screen their cytotoxic activities against human colon (HCT-116) and mantle cell lymphoma (JEKO-1) cancer cell lines. The inhibitory activities of the 22 compounds were *in vitro* determined by means of MTT assay using cisplatin as reference drug. The IC_{50} values, demonstrating the growth inhibition in the presence of tested compounds, were summarized in Table **1**.[30]

Table 1. IC_{50} values (in μM) of tested compounds against HCT-116 and JEKO-1 cell lines

Compd	IC_{50} (μM) [a,b]		Compd	IC_{50} (μM) [a,b]	
	HCT-116	JEKO-1		HCT-116	JEKO-1
1	>150	>150	**10b**	2.987 ± 0.098	12.693 ± 0.370
4	51.433 ± 1.292	9.750 ± 0.178	**10c**	4.637 ± 0.138	15.610 ± 0.399
5	46.101 ± 0.359	55.167 ± 1.558	**10d**	6.620 ± 0.178	16.400 ± 0.773
8	14.822 ± 0.334	31.190 ± 1.747	**10e**	4.040 ± 0.098	7.087 ± 0.351
9a	48.440 ± 2.396	95.917 ± 3.513	**10f**	8.155 ± 0.246	19.593 ± 1.083
9b	3.801 ± 0.228	12.160 ± 0.167	**10g**	19.573 ± 0.698	32.313 ± 1.350

9c	6.502 ± 0.094	9.497 ± 0.337	**11**	27.120 ± 1.134	24.670 ± 1.688
9d	8.200 ± 0.242	11.737 ± 0.619	**12a**	125.713 ± 6.964	15.330 ± 0.330
9e	7.183 ± 0.189	10.863 ± 0.074	**12b**	19.913 ± 1.970	11.360 ± 0.324
9f	9.624 ± 0.372	13.862 ± 0.768	**12c**	19.310 ± 0.818	26.307 ± 0.900
9d'	11.750 ± 0.860	54.357 ± 3.807			
10a	8.570 ± 0.179	18.790 ± 0.842	**Cisplatin**	3.906 ± 0.261	2.743 ± 0.235

[a] Values are the mean of triplicate of three independent experiments

[b] ±SD

From the observed cytotoxic activity data, it has been noticed that all the derivatives of isosteviol showed better cytotoxic activities than their corresponding precursor. More importantly, the inhibitory activities of most compounds were markedly improved as the 1,2,3-triazole subunit was introduced onto the skeleton of isosteviol, which indicated the 1,2,3-triazole fragment exactly played a significant role in inhibiting cancer cell proliferation. Going even further, substituted groups and positions on aromatic ring had a significant effect on cytotoxic activities. Compounds with aldehyde group on aromatic ring exhibited better inhibitory activities than the unsubstituted compounds (**9b**-**9f** vs **9a** and **10b**-**10f** vs **10a**). Oxidation of aldehyde group caused weaker activities to the cancer cell lines (**9d** vs **9d'**).

The colon carcinoma cells were more sensitive to the tested compounds, half of which were found to possess IC_{50} values lower than 10 μM. Especially, the compound **10b** exhibited the most potent anticancer activity with IC_{50} values of 2.987 ± 0.098 μM, which was better than that (3.906 ± 0.261 μM) of positive control cisplatin. In addition, the compound **9b** showed the second-best inhibitory activity against HCT-116 cells with IC_{50} values of 3.801 ± 0.228 μM, which indicates *ortho*-position on aromatic ring has an important effect on cytotoxic activity. To be noteworthiness, the inhibitory activities of isosteviol simultaneously fusing hydroxyl and 1,2,3-triazole subunits were better than that of compounds with only 1,2,3-triazole subunit (**10a** vs **9a, 10b** vs **9b, 10c** vs **9c, 10d** vs **9d, 10e** vs **9e** and **10f** vs **9f**), which illustrates that introduction of hydroxyl group can result in higher inhibitory activity against HCT-116 cells. This conclusion is consistent with previous work by Wu[31] and Zhang[32].

When these synthesized compounds were evaluated against JEKO-1 cells, only compounds **4** and

10e exhibited noteworthy inhibitory activity with IC_{50} values of 9.750 ± 0.178 and 7.087 ± 0.351 μM, respectively, and IC_{50} values of the remaining compounds were greater than 10 μM. Interestingly, the cytotoxic activity against JEKO-1 cells was improved as 1,2,3-triazole ring was introduced onto the 19-carboxyl group to give the derivative **12a**, however, which was inactive to HCT-116 cell line (**11** vs **12a**). It was very disappointing that the inhibitory activities against JEKO-1 cells lacked of regularity, which would adversely influence on the development of satisfactory HQSAR model in follow-up HQSAR study.

Newly synthesized 21 isosteviol derivatives (compounds **4**-**12c**) were chosen for the HQSAR study. Biological data (IC_{50}) of the molecules in Table **1** were used as dependent variable in this study. This whole dataset was segregated in such a way that both training (17 compounds) and test set (4 compounds: **8**, **9d**, **9f** and **10c**) contain high active, moderately active, and low active molecules. The IC_{50} values were converted into pIC_{50} values according to the formula (pIC_{50}= –log IC_{50}).

HQSAR models were generated with seventeen training set compounds using three distinct parameters: fragment distinction, fragment size and hologram length[33]. When three distinct parameters as independent variables were linearly correlated with the pIC_{50} values against JEKO-1 cells as dependent variables by the full cross validated (q^2) partial least square (PLS) leave-one-out (LOO) method, then no satisfactory HQSAR model was obtained due to the results of anticancer activity lacking of regularity. However, predictable and statistical significant models were developed using pIC_{50} values against HCT-116 cells in HQSAR analyses.

Initially, the effects of fragment distinction parameters on the statistical values of models were investigated. The performance of an HQSAR model can be optimized by varying the fragment distinction parameters which determine the compositional and topological structure information encoded in the molecular hologram in terms of atoms (A), bonds (B), connections (C), hydrogens (H), chirality (Ch), donor and acceptor (DA). HQSAR analyses were carried out by using the default hologram length (53, 59, 61, 71, 83, 97, 151, 199, 257, 307, 353 and 401), the default fragment size (4–7) and a combination of one or more fragment distinction parameters. The analysis results were then listed in Table **2**. Among these models, model **9**, which includes atoms, connections, hydrogens, chirality and donor and acceptor (A/C/H/Ch/DA), was chosen as the best

HQSAR model to give significant q^2 and r^2 of 0.503 and 0.855 respectively with component 5 and hologram length 53.

Table 2. HQSAR analysis for various fragment distinction using default fragment size (4–7)

model	Fragment distinction	q^2	SEcv	r^2	SEE	HL	N
1	A/B	0.450	0.413	0.820	0.236	61	5
2	A/B/C	0.489	0.381	0.845	0.210	97	4
3	A/C/H	0.446	0.414	0.795	0.252	53	5
4	A/B/Ch	0.453	0.394	0.832	0.218	71	4
5	A/B/C/Ch	0.477	0.385	0.835	0.217	401	4
6	A/C/H/Ch	0.407	0.394	0.725	0.268	199	3
7	A/C/Ch/DA	0.413	0.426	0.840	0.222	257	5
8	A/B/C/H/Ch	0.448	0.413	0.813	0.241	71	5
9	**A/C/H/Ch/DA**	**0.503**	**0.392**	**0.855**	**0.211**	**53**	**5**
10	A/B/C/H/Ch/DA	0.423	0.422	0.821	0.235	61	5

q^2: LOO cross-validated correlation coefficient. SE_{CV}: cross-validated standard error. r^2: noncross-validated correlation coefficient. SEE: noncross-validated standard error. HL: hologram length. N: optimal number of component. Fragment distinction: A-atoms, B-bonds, C-connections, H-hydrogens, Ch-chirality, DA-donor and acceptor.

In order to optimize statistical results, various fragment sizes were applied with A/C/H/Ch/DA as fragment distinction. The influence of distinct fragment sizes on the statistical parameters was further investigated and the results were summarized in Table **3**. As can be seen from it, although their r^2 values were lower than that of model **5**, the q^2 values of models **3**, **4** and **6** were greater than 0.503. Especially, the fragment size (5–6) led to the best statistical results with q^2 value of 0.544. Therefore, the finally optimal HQSAR model **6** obtained from training set with 17 compounds was established using A/C/H/Ch/DA as fragment distinction, and 5–6 as fragment size with component 5 showing cross-validated q^2 value of 0.544 and conventional r^2 value of 0.848.

Table 3. HQSAR analysis for the influence of fragment sizes using the best fragment distinction (A/C/H/Ch/DA)

model	Fragment size	q^2	SEcv	r^2	SEE	HL	N
1	2-4	0.471	0.424	0.836	0.197	353	6
2	2-5	0.449	0.433	0.835	0.197	353	6
3	3-6	0.528	0.382	0.829	0.216	53	5
4	4-6	0.518	0.386	0.848	0.217	53	5

5	4-7	0.503	0.392	0.855	0.211	53	5
6	**5-6**	**0.544**	**0.376**	**0.848**	**0.217**	**53**	**5**
7	5-7	0.456	0.410	0.834	0.212	53	5
8	5-8	0.242	0.429	0.498	0.349	307	2
9	6-9	0.248	0.428	0.486	0.354	61	2
10	8-9	0.273	0.420	0.501	0.348	61	2

Since the structure encoded with in a two-dimensional fingerprint is directly related to bioactivity of molecule, the HQSAR model is able to predict the activity of new related molecule according to its fingerprint.[34] In virtue of the finally optimal HQSAR model revealing noncross-validated (r^2 = 0.848) and cross-validated (q^2= 0.544) correlation coefficients, these parameters indicated that the model was provided with good internal robustness and predictive power, the predicted pIC_{50} values of training set compounds are summarized in Table **4**. In order to further investigate external predictive power of this model, it was validated by an external test set of four compounds with satisfactory predictive r^2 value of 0.982. The predicted results of test set compounds also listed in Table **4**. As shown in it, all the pIC_{50} residuals of test set compounds were not greater than 0.225 log unit, which is a strong evidence that highly predictive QSAR model was obtained. The correlation between the experimental and predicted activities of both the training set and the test set was graphically displayed in Fig. **3**.

Table 4. Experimental and predicted activities (pIC_{50}) with residual values for 21 compounds

Compd	Exp	Pred	Res	Compd	Exp	Pred	Res
Training				10f	-0.911	-0.677	-0.234
4	-1.711	-1.792	0.081	10g	-1.292	-1.161	-0.131
5	-1.664	-1.757	0.093	11	-1.433	-1.308	-0.125
9a	-1.685	-1.459	-0.226	12a	-2.099	-1.866	-0.233
9b	-0.580	-0.947	0.367	12b	-1.299	-1.514	0.215
9c	-0.813	-0.996	0.183	12c	-1.286	-1.312	0.026
9e	-0.856	-0.903	0.047				
9d'	-1.070	-0.947	-0.123	Test			
10a	-0.933	-1.107	0.174	**8**	-1.171	-1.396	0.225
10b	-0.475	-0.595	0.120	**9d**	-0.914	-1.000	0.086
10d	-0.821	-0.648	-0.173	**9f**	-0.983	-1.029	0.046

10e	-0.606	-0.551	-0.055	**10c**	-0.666	-0.644	-0.022

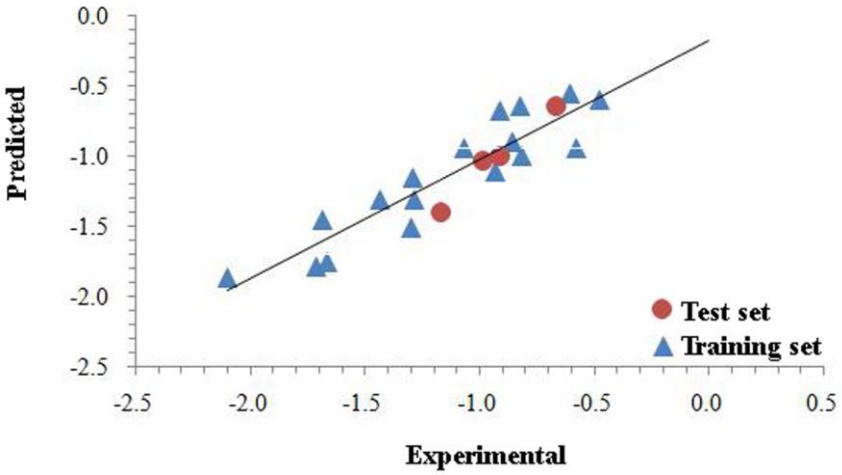


Fig. 3. Plot of experimental values versus predicted values of pIC_{50} of the training and test set

An attractive property of HQSAR technique is that it provides straightforward clues about the individual atomic contributions to the bioactivities using different color codes.[35] The HQSAR analysis results can be graphically displayed in the form of contribution maps, where the color coding of each atom reflects its contribution to the activity of the whole molecule. The colors (red, red orange and orange) at end of the spectrum indicate the negative contribution (NC) to the activity, while ones (yellow, green blue and green) of the other end signify positive contribution (PC) to the activity. The white in the middle domain denotes intermediate contribution (IC) to the activity.

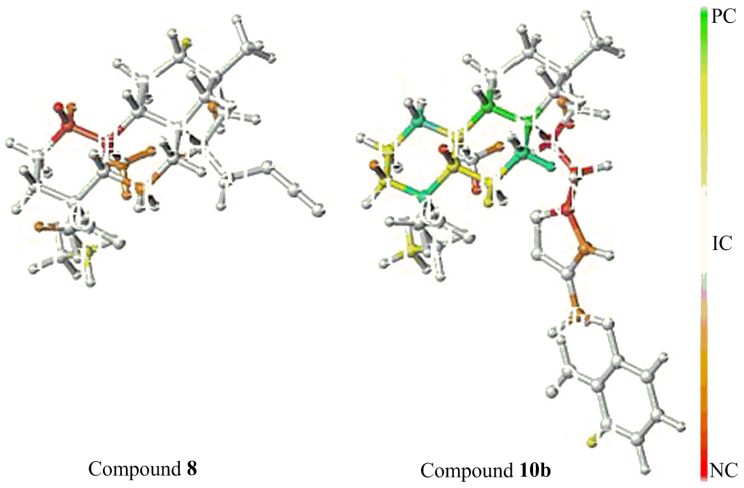


Fig. 4. The HQSAR contribution maps of compounds **8** and **10b**

The individual atomic contribution of the most potent molecule **10b** and its precursor molecule **8** to its molecular bioactivity, resulting from the best HQSAR model, was shown in Fig. **4**. From Fig. **4**, it can be seen that the C–1 and C10-CH_3 of compound **8** were colored the red and orange indicating their negative contributions to the activity, and the remaining structural units were colored the white as intermediate contribution. After introduction of substituted 1,2,3-triazole ring subunit onto the skeleton of isosteviol, although two nitrogen atoms of 1,2,3-triazolering were full of the red and orange colors as unfavorable contribution, A and B rings of compound **10b** were wholly covered by the green and yellow colors reflecting its positive contribution to the activity. This result reinforced the importance of the 1,2,3-triazole ring moiety in establishing the pharmacophore of these inhibitors, and it was deduced that the introduction of substituted 1,2,3-triazole ring may indirectly enhance the interaction between the isosteviol skeleton and the active site of target HCT-116 cell. What's more, regions with intermediate or negative contributions can be identified as the potential targets for the synthesis as well as structure–activity relationship studies.

In conclusion, a series of novel 1,2,3-triazole-linked isosteviol derivatives have been rationally designed and successfully synthesized *via* a Huisgen-click reaction in high yields. Inhibitory activities against HCT-116 and JEKO-1 cancer cell lines of the newly synthesized analogs were investigated *in vitro* using cisplatin as positive control according to the standard procedure. Most of the tested compounds exhibited significant anticancer properties. Especially, compound **10b** (IC_{50} = 2.987 ± 0.098 μM) revealed remarkable anticancer potency compared to that of positive control cisplatin (IC_{50} = 3.906 ± 0.261 μM). On the basis of novel molecular structures and bioactivity data, the HQSAR technique was employed to investigate further structure-activity relationship of these compounds and afforded the robust and good predictive model (q^2 = 0.544, r^2 = 0.848 and r^2_{pred} = 0.982). The results will provide a practical tool for guiding the design and synthesis of novel and more potent isosteviol derivatives containing 1,2,3-triazole moiety in due course.

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1464.

29. Crystallographic data (excluding structure factors) has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 1419389 (compound **9a**). Copies of the data can be obtained, free of charge, on applications to CCDC, 12 Union Road, Cambridge CB2 1EZ. UK (fax: +44 (0) 1223 336033 or E-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).
30. Cytotoxicity assay *in vitro*: HCT-116 and JEKO-1 cells were cultured in RPMI-1640 medium (GIBCO: 31800-022) supplemented with 10% FBS, 100 U/mL of penicillin and 100 mg/mL of streptomycin at 37 ^{o}C in a 5% CO_2 humidified atmosphere. Cell cytotoxicity was assayed by MTT method. Briefly, cells were seeded in 96-well tissue culture plates. After 24 h incubation at 37 ^{o}C, 5% CO_2, removed the culture medium and replaced with fresh medium containing the studied compounds in different concentrations to the wells, and the cells were incubated for another 72 h. Afterwards, the MTT (MP: 102227) solution (0.5 mg/mL) was added and incubated for an additional 4 h. Two hundred microliters of DMSO was added to each well to dissolve the reduced MTT crystals. Optical density of each well was measured at 492/630 nm with enzyme immunoassay instrument (TECAN: Infinite 200 Pro). Then the inhibitory percentage of each compound at various concentrations to the cell proliferation was calculated, and the IC_{50} value was determined.
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33. The HQSAR studies were carried out by using the SYBYL (Tripos Inc., St. Louis, USA) following the standard procedures provided in the manual. HQSAR was run from SYBYL using default parameters: hologram lengths (53, 59, 61, 71, 83, 97, 151, 199, 257, 307, 353 and 401), atom count in fragments (min = 4 and max = 7) were used. The best model was selected on the basis of best crossvalideted r^2. Two options dialog boxes, which are show statistics for model ensemble and retain fragment information for prediticton statistics, were checked. Statistical parameters such as q^2, r^2, number of components were generated.
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Highlights

- ► A series of novel 1,2,3-triazole-linked isosteviol derivatives were synthesized.
- ► Their cytotoxicities were assessed against HCT-116 and JEKO-1cell lines.
- ► Compound **10b** is promising anticancer agent against HCT-116 (IC_{50} = 2.987 ± 0.098 μM).
- ► HQSAR studies revealed good predictive model.

Graphical Abstract:

Syntheses, cytotoxic activity evaluation and HQSAR study of 1,2,3-triazole-linked isosteviol derivatives as potential anticancer agents

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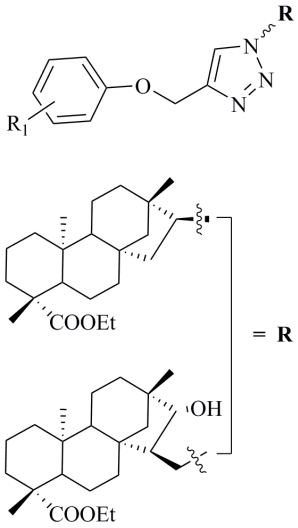

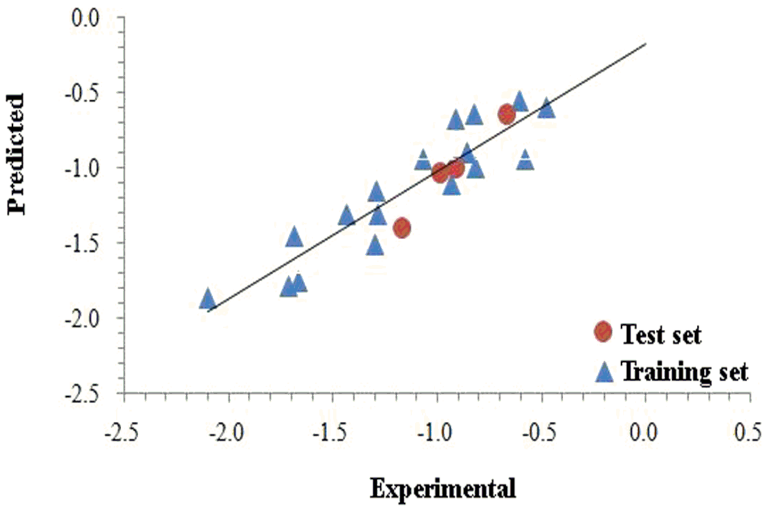

Plot of experimental values versus predicted values of pIC_{50} of the training and test set