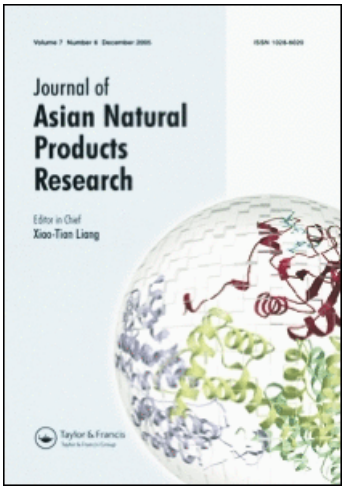

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Synthesis of cytotoxic and antioxidant Schiff's base analogs of aloin

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ORIGINAL ARTICLE

Synthesis of cytotoxic and antioxidant Schiff's base analogs of aloin

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Aloin (10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9(10H)-anthracenone), a bioactive compound in *Aloe vera*, although known to have an anticancer effect, has not been used in current drug research. Optimization of the lead structure could enhance the utility of this compound. Hence, aloin was modified using natural amino acids to produce Schiff's base, a potential pharmacophore, and its corresponding aglycones. The synthetic derivatives exhibited significant enhancement in their efficacy toward antioxidant (DPPH radical scavenging) and cytotoxic activities than those of the parent compound, aloin showing promise for application in cancer treatment.

Keywords: Schiff's base; aloin; semi-synthetic modification; activity studies

1. Introduction

Cancer is the second leading cause of death in the world. Almost all synthetic agents used in cancer therapy are known to be toxic and produce severe damages to normal cells. Hence, chemoprevention of these toxic agents is one approach to minimize the effect of the cancerous cells. Naturally occurring antioxidants found in medicinal plants are good alternatives to these chemical anticancer agents. Many studies reveal that the naturally occurring polyphenolic compounds can be utilized for the prevention and treatment of cancer [1–6]. One such naturally occurring medicinal plant is *Aloe vera.*

A. vera (Asphodelaceae) is a well-known, widely distributed herb found all over the Asian countries [7]. This plant has been used from time immemorial for various applications in medical, cosmetic, and nutraceutical areas [8,9]. Many biological activities including analgesic, antiviral, antifungal, antibacterial, and anti-inflammatory effects have been attributed to this plant. Aloin (10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9(10H)-anthracenone), an active compound obtained from *A. vera* species (Figure 1), was confirmed to exhibit an anticancer effect. However, scientifically proven [10,11] information available is limited for the *in vivo* supplements of aloin-based drugs in cancer research. Hence, synthetic modifications are necessary to project their efficacy and bioavailability for antitumor studies. Of the possible modifications of the functional groups present in the lead molecule aloin, products derived mainly from the reaction of the keto group and amino acids are of interest since azomethine linkage is a necessary unit in all active drugs [12,13]. Also, the glucose unit, which contributes to the hydrophilicity, can

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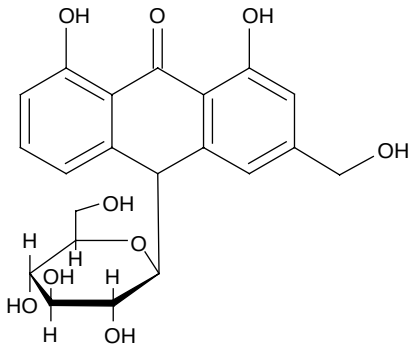


Figure 1. The structure of aloin.

be cleaved to produce the aglycones of the corresponding Schiff's bases.

In this study, we report the synthesis of 18 Schiff's bases (glycone and aglycone products) and their antioxidant and cytotoxic activities.

2. Results and discussion

The Schiff's bases were obtained by refluxing aloin with the corresponding amino acids in a methanol medium, using sulfuric acid as the catalyst (Figure 2). The glycoside linkage was intact in most of the cases except in the case of amino acids such as glycine, alanine, and phenyl alanine, wherein the reaction resulted in the cleavage of the glucose moiety. These reactions were sluggish requiring long hours of refluxing (12 h). Therefore, in order to prepare the glycosides from these amino acids, the reaction rate has to be enhanced. Interestingly, this was done by employing microwave irradiation for 30–45 min when these Schiff's bases could be synthesized without the cleavage of glucoside. In other cases, the deglycosylated Schiff's bases were made from the corresponding glycosylated derivatives by oxidative hydrolysis using Jones reagent (Figure 3).

It is interesting to note that the structures (Figure 4) represent systematic variation of the alkyl substituent from methyl to sterically hindering isopropyl and isobutyl groups, and polar substituents such as hydroxyl and thiol groups.

The cytotoxic assay using brine shrimp lethality test was carried out for the parent compound aloin and its aglycone and all Schiff's bases at different concentrations (100–400 μM) to obtain the MIC. From the MIC, the ED_{50} values were obtained using Finney's Probit–Logit analysis [14]. The application of the structure–activity relationship indicated two distinct features: (1) the presence of the glycoside unit and (2) the alkyl chain variation. Interestingly, the glycosides show better cytotoxicity than the corresponding aglycones. The results are shown in Table 1. The presence of branching in the alkyl groups enhances the activity in the following

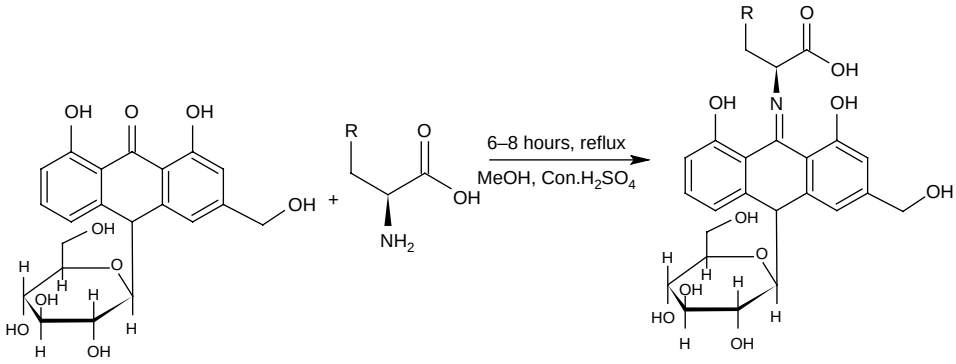


Figure 2. Synthesis of glycosylated Schiff's bases.

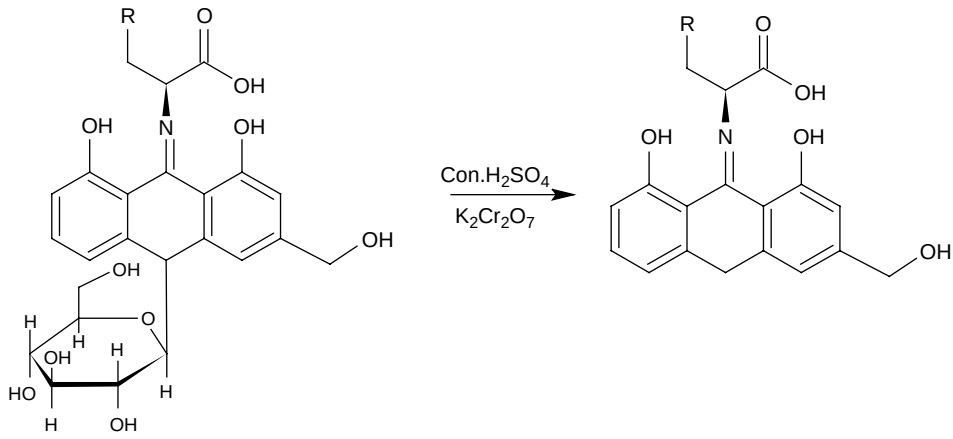
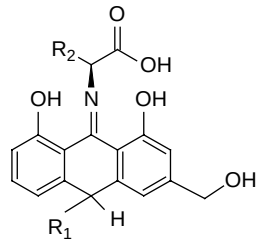


Figure 3. Synthesis of deglycosylated Schiff's bases.



Compound	Glycones (1)	Aglycones (2)	Corresponding amino acids used
A	R_1 = glu, R_2 = H	R_1 = H, R_2 = H	Glycine
B	R_1 = glu, R_2 = CH_3	R_1 = H, R_2 = CH_3	Alanine
C	R_1 = glu, R_2 = $C_6H_5CH_2$	R_1 = H, R_2 = $C_6H_5CH_2$	Phenyl alanine
D	R_1 = glu, R_2 = $CH_2CH(CH_3)_2$	R_1 = H, R_2 = $CH_2CH(CH_3)_2$	Leucine
E	R_1 = glu, R_2 = $CH(CH_3)CH_2CH_3$	R_1 = H, R_2 = $CH(CH_3)CH_2CH_3$	Isoleucine
F	R_1 = glu, R_2 = CH_2SH	R_1 = H, R_2 = CH_2SH	Cysteine
G	R_1 = glu, R_2 = $CH_2CH_2SCH_3$	R_1 = H, R_2 = $CH_2CH_2SCH_3$	Methionine
H	R_1 = glu, R_2 = $CH(CH_3)_2$	R_1 = H, R_2 = $CH(CH_3)_2$	Valine
I	R_1 = glu, R_2 = CH–OH–CH_3	R_1 = H, R_2 = CH–OH–CH_3	Threonine

Figure 4. List of amino acids used.

Table 1. The comparison of cytotoxicities of synthesized compounds.

Compound	ED_{50} (μM)	
	Glycones (I)	Aglycones (II)
Aloin	95.17	143.21
A	407.84	1028.33
B	130.02	338.64
C	311.64	334.67
D	32.06	72.4
E	35.24	245.62
F	10.16	423.01
G	403.93	835.46
H	19.13	145.11
I	27.60	338.64

order:

isopropyl > secondary butyl > isobutyl > benzyl.

The comparison points out that most polar groups have significant improvement in the cytotoxic activity. This is also reflected in the polar substituent in the alkyl chain: SH and OH.

The free radical scavenging activity of Schiff's bases including both glycones and aglycones was tested by their ability to bleach the stable radical DPPH. The activity was monitored by following the absorption at 517 nm in a visible spectrophotometer. In the presence of any free radical scavenger, this odd electron pairs up and causes the diminishing of absorption band which is proportional to the number of electrons taken up.

The activity was studied at different concentrations (0.1–0.4 mmol) for each compound. The variations in activity for glycones and aglycones are represented in graphs (Figures 5 and 6). All the molecules showed a typical scavenging activity with little variation at higher concentrations. From the data, the antioxidant activity was calculated as a percentage with reference to gallic acid as the standard. The values are given in Table 2. The IC_{50} values for each of the Schiff's bases are calculated and presented in Table 3.

The striking feature observed from this study is that the glycosylated Schiff's bases possess slightly increased activity than the aglycones. However, the variation is not significant, indicating that the glucose unit does not contribute to the antioxidant effect. The alkyl substituents have shown strikingly the same gradation in the activity as observed for the cytotoxicity as follows:

isopropyl = secondary butyl > isobutyl > benzyl.

The presence of polar groups in the alkyl chain also showed a similar activity trend.

In conclusion, we find that the presence of the functional groups such as glycoside, isopropyl, and thiol significantly contributes to the bioactivity of

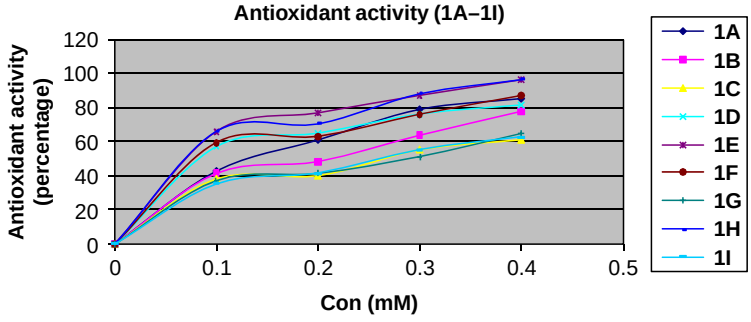


Figure 5. Antioxidant activities of glycone Schiff's bases.

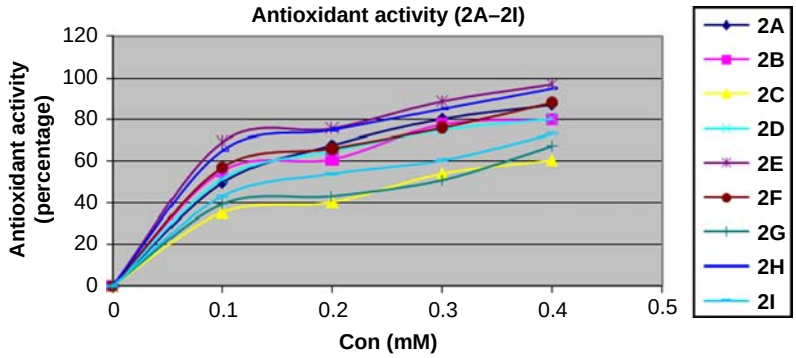


Figure 6. Antioxidant activities of deglycosylated Schiff's bases.

the lead molecule, aloin. In view of their antioxidant and cytotoxic activities, the synthetic Schiff's base analogs have potential in cancer treatment.

3. Experimental

Fresh *A. vera* was collected from a farm in Tirupathi hills and processed as per the literature method to obtain aloin as a yellow solid [15]. Melting points were determined using a Raaga Industries melting point apparatus and are uncorrected. Mass spectrometry was performed in JEOL GC mate in IITM. HPLC were performed on a Shimadzu LC-10 ATVP high-pressure pump and a C18 Luna 5 column (250 × 4.60 mm) and the peaks were detected at 215 nm (SPD-10 AVP UV–vis detector), the mobile phase being the methanol–water system (95:5) at a flow rate of 0.5 ml/min. The purity was confirmed by HPLC and NMR. The NMR spectra were recorded at 27°C with a Bruker 500 MHz spectrometer using TMS as the internal standard and CD_3OD as the solvent. The synthesized compounds were evaluated for biological activities such as cytotoxicity and antioxidant activity.

Table 2. The comparison of antioxidant potential of synthesized compounds.

Compound	Antioxidant potential (%)	
	Glycones (I)	Aglycones (II)
A	62.58	73.13
B	77.55	80.17
C	63.2	68.9
D	81.63	80.43
E	96.59	97.13
F	87.07	89.35
G	64.62	67.31
H	96.59	95.12
I	85.71	87.1

3.1 *Synthesis of glycosylated Schiff's bases*

In a typical procedure, weighed quantities of aloin and amino acid (Sigma Aldrich's Standard, Buchs, Germany) in the ratio of 1:1 were allowed to reflux in a methanol medium with a catalytic amount of concentrated sulfuric acid. The reaction was monitored by TLC (60–120 mesh; Sigma Aldrich). After 6–8 h, methanol was distilled off in a rotary evaporator and the residue was subjected to column chromatography. Pure Schiff's base compound was eluted in an 8:2 methanol–chloroform mobile phase. The purity, yield, and melting point of the synthesized glycones and aglycones are given in Tables 4 and 5.

3.1.1 *Compound **1A***

IR (KBr) ν_{max}: 3200, 1673, 699.3 cm^{-1}; ^1H NMR (CH_3OH-d_4) δ in ppm: 11.00 (br, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2H, phenolic), 4.79 (s, 2, CH_2 of primary

Table 3. Radical scavenging activities of synthesized products.

Compound	IC_{50} (μM)	
	Glycones (I)	Aglycones (II)
Aloin	132	145
A	95	85
B	205	98
C	256	268
D	258	151
E	96	88
F	98	98
G	235	271
H	95	110
I	240	179

OH), 4.51 (s, 2, CH_2 in glycine), 4.37 (s, 2, H on 10-C), 2.00 (s, 1, primary OH), 4.44, 3.40, 3.49, 3.76, 3.79 (br m, 5H, glucose unit), 3.54 (br s, 2H, CH_2 of glucose), 2.00 (s, 4H, OH of glucose); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 178.1 (acidic C of amino acid), 162.7 (8-C), 162.2 (1-C), 152.9 (9-C), 146.5 (3-C), 135.5 (6-C), 121.6 (5-C), 119.7 (4-C), 114.1 (7-C), 112.6 (2-C), 63.5 (11-C), 51.4 (C attached to N and carbonyl group), 40.5 (10-C), 85.3, 72.1, 78.2, 70.5, 82.1, 63.0 (glucose C); MS: *m/z* 473.6 $[M]^+$, 244.6.

3.1.2 Compound **1B**

IR (KBr) ν_{max}: 3333, 1603, 1021 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 11.00 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2H, phenolic), 4.79 (s, 2, CH_2 of primary OH), 4.37 (s, 2, H on 10-C), 4.18 (d, 3, $J = 11$ Hz, methyl in alanine), 2.00 (s, 1 primary OH), 1.48 (q, 1, $J = 13$ Hz, CH in alanine), 4.50, 3.38, 3.49, 3.76, 3.79 (br m, 5H, glucose unit), 3.54 (br s, 2H, CH_2 of glucose), 2.40 (s, 4H, OH of glucose); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 177.5 (acidic C of amino acid), 162.7 (8-C), 161.8 (1-C), 150.2 (9-C), 148.6 (3-C), 135.4 (6-C), 120.3 (5-C), 119.6 (4-C), 112.9 (7-C), 112.5 (2-C), 64.1 (11-C), 56.5 (C attached to N and carbonyl group), 39.2 (10-C), 18.1 (methyl C of alanine), 85.0, 72.7, 78.2, 70.4, 82.1, 63.0 (glucose C); MS: *m/z* 489 $[M]^+$, 249.

Table 4. Synthesis of the glycosylated product.

Compound **1**	Yield (%)	Melting point (°C)
A	55	128
B	52	121
C	67	174
D	58	117
E	48	144
F	57	140
G	48	157
H	55	148
I	44	Decomposed

3.1.3 Compound **1C**

IR (KBr) ν_{max}: 3433, 2492, 1655, 1014 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 11.00 (br s, 1, acidic proton), 7.40, 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2H, phenolic), 4.79 (s, 1, CH_2 of primary OH), 4.37 (s, 2, H on 10-C), 4.35 (t, 1, $J = 13.0$ Hz, H linked to C via N and carbonyl C), 3.38, 3.13 (CH_2 of phenyl alanine), 2.00 (s, 1, primary OH), 4.40, 3.30, 3.49, 3.77, 3.79 (br m, 5H, glucose unit), 3.54 (br s, 2H, CH_2 of glucose), 2.10 (s, 4H, OH of glucose); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 176.7 (acidic C of amino acid), 171.2 (1-C), 171.1 (8-C), 138.2 (9-C), 130.4 (7-C), 130.3 (6-C), 129.91 (4-C), 129.5 (5-C), 128.3 (3-C), 127.8 (2-C), 68.6 (11-C), 56.6 (C attached to N and carbonyl group), 38.5 (10-C), 38.5 (CH of phenyl alanine), 127.8, 126.0, 128.7, 129.7, 139.5 (aromatic carbons, phenyl group of alanine), 85.1, 72.3, 78.1, 70.1, 82.3, 63.0 (glucose C); MS: *m/z* 415 $[M]^+$, 245, 204.

3.1.4 Compound **1D**

IR (KBr) ν_{max}: 3200, 2927, 1603 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 11.00 (br s, 1, acidic proton), 10.70 (s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br

Table 5. Synthesis of the deglycosylated product.

Compound **2**	Yield (%)	Melting point (°C)
A	43	139
B	48	147
C	68	189
D	49	154
E	65	162
F	62	157
G	57	154
H	55	144
I	68	138

m, 5H, aromatic protons), 5.01 (br s, 2, phenolic), 4.80 (s, 1, CH_2 of primary OH), 4.00 (t, 1, $J = 14$ Hz, H linked to C via N and carbonyl C), 3.80 (s, 1, H on 10-C), 2.30 (s, 1, primary OH), 1.84 (m, 4, CH_2 of leucine), 1.83 (m, 1, CH on leucine), 1.01 (d, $J = 11.0$ Hz, 6, methyl H on leucine), 4.40, 3.40, 3.49, 3.76, 3.79 (br m, 5H, glucose unit), 3.50 (br s, 2H, CH_2 of glucose), 2.00 (s, 4H, OH of glucose); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 176.7 (acidic C of amino acid), 161.2 (1-C), 161.2 (8-C), 153.6 (9-C), 143.5 (3-C), 135.8 (6-C), 127.8 (4-C), 127.4 (5-C), 115.4 (7-C), 111.1 (2-C), 63.6 (11-C), 55.0 (C attached to N and carbonyl group), 36.8 (10-C), 26.7 (CH_2 of leucine), 23.1 (CH of leucine), 21.2 (methyl C of leucine), 85.3, 72.1, 78.2, 70.1, 82.0, 63.0 (glucose C); MS: *m/z* 505 $[M]^+$, 496, 245.5

3.1.5 *Compound **1E***

IR (KBr) ν_{max}: 3200, 2922, 1640, 1023 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 10.60 (br s, 1, acidic proton), 7.25, 6.78, 6.71, 6.54, 6.53 (br m, 5H, aromatic protons), 4.90 (br s, 2, phenolic), 4.80 (s, 2, CH_2 of primary OH), 3.80 (s, 1, H on 10-C), 2.50 (s, 1, primary OH), 2.00 (m, 1, CH on isoleucine), 1.00, 0.96 (methyl H on isoleucine), 4.44, 3.79, 3.76, 3.49, 3.54 (br m, 5H, glucose unit), 3.40 (br s, 2H, CH_2 of glucose), 2.00 (s, 4H, OH of glucose); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 178.9 (acidic C of amino acid), 162.5 (8-C), 161.2 (1-C), 152.2 (9-C), 143.3 (3-C), 135.8 (6-C), 128.7 (5-C), 121.5 (4-C), 115.8 (7-C), 111.2 (2-C), 68.7 (11-C), 66.0 (CH of isoleucine), 56.1 (C attached to N and carbonyl group), 43.9 (d, 1, H linked to C via N and carbonyl C), 36.8 (10-C), 25.6 (CH_2 of isoleucine), 24.3 (CH of leucine), 16.8, 11.8 (methyl C of leucine), 83.1, 82.6, 79.1, 74.6, 73.1, 62.8 (glucose C); MS: *m/z* 519 $[M]^+$, 245.

3.1.6 *Compound **1F***

IR (KBr) ν_{max}: 3200, 2922, 1619, 1024 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 10.80 (br s, 1, acidic proton), 7.30, 6.80, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.40 (br s, 2, phenolic), 5.01 (t, $J = 13.0$ Hz, 2, H linked to C via N and carbonyl C), 4.70 (s, 1, CH_2 of primary OH), 3.80 (s, 1, H on 10-C), 2.60 (s, 1, primary OH), 1.50 (s, 1, H attached to S), 4.30, 3.79, 3.76, 3.54, 3.50 (br m, 5H, glucose unit), 3.49 (br s, 2H, CH_2 of glucose), 2.30 (s, 4H, OH of glucose); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 179.0 (acidic C of amino acid), 161.7 (1-C), 161.2 (8-C), 152.1 (9-C), 144.6 (3-C), 125.2 (6-C), 120.7 (5-C), 120.2 (4-C), 115.7 (7-C), 112.4 (2-C), 70.7 (C attached to N and carbonyl group), 68.2 (11-C), 37.8 (10-C), 23.4 (CH_2 attached to SH), 84.1, 80.7, 79.1, 70.1, 70.3, 63.1 (glucose C); MS: *m/z* 529 $[M]^+$, 245.5.

3.1.7 *Compound **1G***

IR (KBr) ν_{max}: 3200, 2922, 1636, 1129 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 10.60 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 4.79 (s, 2, CH_2 of primary OH), 4.00 (t, 1, $J = 14$ Hz, H linked to C via N and carbonyl C), 3.80 (s, 1, H on 10-C), 2.70 (s, 1, primary OH), 2.44 (CH_2 of methionine), 2.25, 2.09 (s, 3, methyl H on methionine), 4.44, 3.79, 3.76, 3.54, 3.49 (br m, 5H, glucose unit), 3.40

(br s, 2H, CH_2 of glucose), 2.00 (s, 4H, OH of glucose); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 178.9 (acidic C of amino acid), 161.2 (8-C), 161.2 (1-C), 152.6 (9-C), 144.3 (3-C), 134.6 (6-C), 120.3 (5-C), 119.6 (4-C), 110.1 (2-C), 111.6 (7-C), 69.3 (11-C), 60.3 (C attached to N and carbonyl group), 41.6 (10-C), 31.1 (CH_2 of methionine), 30.1, 18.1 (methyl C of methionine), 84.4, 81.2, 78.5, 72.2, 71.1, 63.0 (glucose C); MS: *m/z* 529 $[M]^+$, 245.5.

3.1.8 Compound **1H**

IR (KBr) ν_{max}: 3200, 2921, 1635, 1083 cm^{-1}; ^1H NMR (CH_3OH-d_4) δ in ppm: 10.90 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 4.65 (s, 2, CH_2 of primary OH), 4.35 (d, 1, $J = 11.0$ Hz, H linked to C via N and carbonyl C), 3.80 (s, 1, H on 10-C), 2.91 (s, 1, primary OH), 2.20 (m, 1, CH on valine), 1.01 (d, 6, methyl H on valine), 4.53, 3.79, 3.76, 3.49, 3.32 (br m, 5H, glucose unit), 3.30 (br s, 2H, CH_2 of glucose), 2.20 (s, 4H, OH of glucose); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 178.3 (acidic C of amino acid), 162.3 (8-C), 162.1 (1-C), 152.4 (9-C), 145.4 (3-C), 120.4 (4-C), 119.4 (5-C), 115.1 (6-C), 114.4 (7-C), 110.4 (2-C), 69.3 (11-C), 56.5 (C attached to N and carbonyl group), 38.2 (10-C), 38.2 (CH of valine), 20.1, 20.7 (methyl C of valine), 83.2, 80.6, 79.7, 71.1, 70.6, 64.3 (glucose C); MS: *m/z* 547 $[M]^+$, 245.

3.1.9 Compound **1I**

IR (KBr) ν_{max}: 3200, 2922, 163, 1029 cm^{-1}; ^1H NMR (CH_3OH-d_4) δ in ppm: 10.70 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 4.79 (s, 2, CH_2 of primary OH), 3.80 (s, 1, H on 10-C), 3.70 (d, 1, $J = 11.0$ Hz, H linked to C via N and carbonyl C), 3.20 (s, 1, secondary OH on threonine), 2.80 (s, 1, primary OH), 1.21 (methyl H on threonine), 4.40, 3.79, 3.76, 3.54, 3.49 (br m, 5H, glucose unit), 3.40 (br s, 2H, CH_2 of glucose), 2.00 (s, 4H, OH of glucose); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 177.5 (acidic C), 161.8 (1-C), 161.6 (8-C), 154.4 (9-C), 145.4 (3-C), 123.5 (6-C), 121.2 (5-C), 120.9 (4-C), 113.5 (7-C), 110.4 (2-C), 68.7 (11-C), 67.0 (CH of threonine), 62.7 (CH attached to N), 37.1 (10-C), 25.4 (CH of threonine), 19.8 (methyl C of threonine), 85.2, 82.3, 78.7, 72.3, 70.0, 63.0 (glucose C); MS: *m/z* 517.4 $[M]^+$, 245.5, 204.

3.2 Synthesis of deglycosylated Schiff's bases

The glycosylated Schiff's bases of aloin are taken in methanol and refluxed with stoichiometric quantities of Jones reagent (concentrated sulfuric acid and potassium dichromate). The reaction was monitored by TLC (60–120 mesh; Sigma Aldrich). After 3–4 h, methanol was distilled off and the product was obtained as aglycones.

3.2.1 Compound **2A**

IR (KBr) ν_{max}: 3200, 1637 cm^{-1}; ^1H NMR (CH_3OH-d_4) δ in ppm: 11.0 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 4.79 (s, 2, CH_2 of primary OH), 4.51 (s, 2, CH_2 in glycine), 4.37 (s, 2, H on 10-C), 2.00 (s, 1, primary OH); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 178.3 (acidic C of amino acid), 162.1 (8-C), 162.3 (1-C), 152.0 (9-C), 146.7 (3-C), 135.1 (6-C), 121.7 (5-C), 119.9 (4-C), 112.1 (2-C), 114.8 (7-C), 63.7 (11-C), 51.7 (C attached to N and carbonyl group), 40.3 (10-C); MS: *m/z* 310 $[M]^+$, 245.

3.2.2 Compound **2B**

IR (KBr) ν_{max}: 3233, 2237, 1618 cm^{-1}; ^1H NMR (CH_3OH-d_4) δ in ppm: 11.0 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 4.79 (s, 2, CH_2 of primary OH),

4.37 (s, 2, H on 10-C), 4.18 (d, 3, $J = 11.0$ Hz, methyl in alanine), 2.00 (s, 1, primary OH), 1.48 (q, $J = 15.0$ Hz, 1, CH in alanine); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 177.5 (acidic C of amino acid), 162.8 (8-C), 161.8 (1-C), 150.2 (9-C), 148.5 (3-C), 135.5 (6-C), 120.3 (5-C), 119.6 (4-C), 112.2 (2-C), 112.9 (7-C), 64.9 (11-C), 56.5 (C attached to N and carbonyl group), 39.3 (10-C), 18 (methyl C of alanine); MS: *m/z* 323 $[M]^+$, 247, 227.

3.2.3 *Compound* ***2C***

IR (KBr) ν_{max}: 3333, 2237, 1618 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 11.0 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 7.07, 7.07, 7.07, 7.23, 7.23, (br m, 5H, aromatic protons of phenyl alanine), 4.79 (s, 1, CH_2 of primary OH), 4.37 (s, 2, H on 10-C), 4.35 (t, 1, $J = 13.0$ Hz, H linked to C via N and carbonyl C), 3.38, 3.13 (CH_2 of phenyl alanine), 2.00 (s, 1, primary OH); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 176.7 (acidic C of amino acid), 171.8 (8-C), 171.2 (1-C), 129.2, 129.2, 125.6, 121.6, 121.6 (aromatic carbons of the phenyl group of alanine), 151.4 (C attached to O of the phenyl alanine ring), 138.2 (9-C), 130.4 (7-C), 130.3 (6-C), 129.9 (4-C), 129.7, 129.5 (5-C), 128.3 (3-C), 128.0, 127.8 (2-C), 127.5, 126.0, 38.5 (10-C), 68.6 (11-C), 56.6 (C attached to N and carbonyl group), 38.5 (CH_2 of phenyl alanine); MS: *m/z* 388 $[M]^+$, 245, 204.

3.2.4 *Compound* ***2D***

IR (KBr) ν_{max}: 3333, 2237, 1618 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 10.70 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 4.80 (s, 1, CH_2 of primary OH), 4.00 (t, 1, $J = 14.0$ Hz, H linked to C via N and carbonyl C), 3.80 (s, 1, H on 10-C), 2.30 (s, 1, primary OH), 1.84 (m, 4, CH_2 of leucine), 1.83 (m, 1, CH on leucine), 1.01 (d, $J = 11.0$ Hz, 6, methyl H on leucine); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 176.7 (acidic C of amino acid), 161.5 (1-C), 161.2 (8-C), 153.3 (9-C), 143.7 (3-C), 135.8 (6-C), 127.4 (5-C), 127.2 (4-C), 115.7 (7-C), 111.8 (2-C), 63.9 (11-C), 55.2 (C attached to N and carbonyl group), 36.8 (10-C), 26.7 (CH_2 of leucine), 23.8 (CH of leucine), 21.2 (methyl C of leucine); MS: *m/z* 334 $[M]^+$, 245, 204.

3.2.5 *Compound* ***2E***

IR (KBr) ν_{max}: 3300, 2963, 1636 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 10.60 (br s, 1, acidic proton), 7.25, 6.78, 6.71, 6.54, 6.53 (br m, 5H, aromatic protons), 4.90 (br s, 2, phenolic), 4.80 (s, 2, CH_2 of primary OH), 3.80 (s, 1, H on 10-C), 2.50 (s, 1, primary OH), 2.00 (m, 1, CH on isoleucine), 1.00, 0.96 (methyl H on isoleucine); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 178.9 (acidic C of amino acid), 162.5 (8-C), 161.2 (1-C), 152.8 (9-C), 143.3 (3-C), 135.8 (6-C), 128.7 (5-C), 121.2 (4-C), 115.7 (7-C), 111.2 (2-C), 68.7 (11-C), 66.0 (CH of isoleucine), 56.2 (C attached to N and carbonyl group), 44.0 (C of isoleucine attached to N), 36.8 (10-C), 25.6 (CH_2 of isoleucine), 24.0 (CH of leucine), 16.8 (methyl C of leucine), 11.8 (methyl attached to CH_2 of isoleucine); MS: *m/z* 336.3 $[M]^+$, 245.9, 177.7.

3.2.6 *Compound* ***2F***

IR (KBr) ν_{max}: 3300, 2963, 1636 cm^{-1}; ^{1}H NMR (CH_3OH-d_4) δ in ppm: 10.80 (br s, 1, acidic proton), 7.30, 6.80, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.40 (br s, 2, phenolic), 5.01 (t, $J = 14.0$ Hz, 2, H linked to C via N and carbonyl C), 4.70 (s, 1, CH_2 of primary OH), 3.80 (s, 1, H on 10-C), 2.60 (s, 1, primary OH), 1.50 (s, 1, H attached to S); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 179.1 (acidic C of amino acid), 161.4 (8-C), 161.3 (1-C), 152.8 (9-C), 144.8 (3-C), 125.8 (6-C), 120.2 (4-C), 120.1 (5-C), 115.7 (7-C), 112.5 (2-C), 70.4 (C attached to N and carbonyl group), 68.1

(11-C), 37.3 (10-C), 23.4 (CH_2 attached to SH); MS: *m/z* 366.3 $[M]^+$, 245.9, 177.7.

3.2.7 Compound **2G**

IR (KBr) ν_{max}: 3324, 2922, 1648 cm^{-1}; ^1H NMR (CH_3OH-d_4) δ in ppm: 10.6 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 4.79 (s, 2, CH_2 of primary OH), 4.00 (t, 1, $J = 13.0$ Hz, H linked to C via N and carbonyl C), 3.80 (s, 1, H on 10-C), 2.70 (s, 1, primary OH), 2.44 (CH_2 of methionine), 2.25, 2.09 (s, 3, methyl H on methionine); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 178.9 (acidic C of amino acid), 161.7 (1-C), 161.2 (8-C), 152.7 (9-C), 144.2 (3-C), 134.1 (6-C), 120.2 (5-C), 119.8 (4-C), 110.3 (2-C), 111.0 (7-C), 69.5 (11-C), 60.6 (C attached to N and carbonyl group), 41.9 (10-C), 31.0 (CH of methionine), 30.0, 18.0 (methyl C of methionine); MS: *m/z* 356 $[M]^+$, 245, 220.

3.2.8 Compound **2H**

IR (KBr) ν_{max}: 3324, 2922, 1648 cm^{-1}; ^1H NMR (CH_3OH-d_4) δ in ppm: 10.90 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 4.65 (s, 2, CH_2 of primary OH), 4.35 (d, 1, $J = 11.0$ Hz, H linked to C via N and carbonyl C), 3.80 (s, 1, H on 10-C), 2.90 (s, 1, primary OH), 2.20 (m, 1, CH on valine), 1.01 (d, $J = 11.0$ Hz, 6, methyl H on valine); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 178.3 (acidic C of amino acid), 162.4 (8-C), 162 (1-C), 152.1 (9-C), 145.3 (3-C), 120.8 (4-C), 119.2 (5-C), 115.7 (6-C), 114.6 (7-C), 110.1 (2-C), 69.5 (11-C), 56.4 (C attached to N and carbonyl group), 38.9 (CH of valine), 38.3 (10-C), 20.0, 20.0 (methyl C of valine); MS: *m/z* 385 $[M]^+$, 245, 220.

3.2.9 Compound **2I**

IR (KBr) ν_{max}: 3339, 2973, 1637 cm^{-1}; ^1H NMR (CH_3OH-d_4) δ in ppm: 10.7 (br s, 1, acidic proton), 7.23, 6.78, 6.71, 6.65, 6.58 (br m, 5H, aromatic protons), 5.00 (br s, 2, phenolic), 4.79 (s, 2, CH_2 of primary OH), 3.80 (s, 1, H on 10-C), 3.70 (d, 1, $J = 11$ Hz, H linked to C via N and carbonyl C), 3.20 (s, 1, secondary OH on threonine), 2.80 (s, 1, primary OH), 1.21 (methyl H on threonine); ^{13}C NMR (CH_3OH-d_4) δ in ppm: 177.5 (acidic C), 161.8 (1-C), 161.6 (8-C), 154.4 (9-C), 145.4 (3-C), 123.2 (6-C), 121.0 (5-C), 120.9 (4-C), 113.5 (7-C), 110.4 (2-C), 68.7 (11-C), 67.0 (CH of threonine), 62.7 (CH attached to N), 37.1 (10-C), 19.8 (methyl C of threonine); MS: *m/z* 356 $[M]^+$, 245, 213.

3.3 Cytotoxic assay

The nauplii of brine shrimp *Artemia salina* were hatched from cysts that were obtained from the National Institute of Ocean Technology, Chennai. Cyst hatching was initiated 24 h prior to the start of the toxicity test. Typically, the cyst was taken in a standard seawater with a salinity of 35 g/l prepared in the laboratory and exposed to a light source (1000–4000 lux) for 24 h at 25°C. The toxicity experiment was performed in a multiwell plate, each well containing 5 ml of test solution prepared with the respective aloin analog and 10 larvae [16]. A control was maintained in which the larvae were taken in a standard seawater. The multiwell plate was maintained at 25°C for 24 h for the toxicity tests. After the incubation was completed, the number of dead *Artemia* species for each compound at different concentrations was counted and the percentage mortality was evaluated. The experiments were done at different concentrations. All toxicity tests were done in duplicate.

3.4 Antioxidant activity using DPPH radical

To measure the antioxidant potential, experiments were carried out according

to the standard method. Solutions (1 mM) of DPPH radical in methanol were prepared and 1 ml of it was mixed with 3 ml of the sample solution in ethanol. After 30 min, the absorbance was measured at 517 nm [17]. A decrease in the absorption of the DPPH solution indicates an increase in the DPPH radical scavenging activity. The results were compared with respect to gallic acid as the standard.

3.5 Statistical analysis

Determination of equivalent dosage for cytotoxic studies was calculated using Finney's Probit–Logit analysis.

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