Synthesis and Biological Activity of Schiff Base Series of Valproyl, *N*-Valproyl Glycinyl, and *N*-Valproyl-4-aminobenzoyl Hydrazide Derivatives

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Series of Schiff bases of valproic acid hydrazide, *N*-valproylglycine hydrazide and *N*-valproyl-4-aminobenzoyl hydrazide derivatives were synthesized and characterized by IR, NMR (^{1}H- and ^{13}C-NMR) and elemental analysis. The prepared compounds were evaluated in transgenic zebrafish embryos (Tg: *flil-1*: enhanced green fluorescent protein (EGFP)) for antiangiogenic activity and in HepG2 liver carcinoma cell line for anti cancer activity. The Schiff bases of *N*-valproylglycine hydrazide derivatives were most potent in term of suppressing angiogenic blood vessels formation and anticancer activity at very low concentration, followed by the Schiff bases of valproic acid hydrazide derivatives which exhibited moderate activity, while the Schiff bases of *N*-valproyl-4-aminobenzoyl hydrazide derivatives, ethyl 4-(2-propylpentanamido)benzoate (VABE) and *N*-(4-(hydrazinecarbonyl)phenyl)-2-propylpentanamide (VABH) in contrast exhibited pro-angiogenic activity and weaker anticancer activity which mean that these derivatives targeted a common signaling pathway in term of affecting the blood vessels formation.

Key words valproic acid; valproic acid hydrazide; Schiff base; antiangiogenic activity; anticancer activity

Valproic acid (VPA, **1**) is a widely used antiepileptic agent that is undergoing clinical evaluation for anticancer therapy. Given the remarkable value of valproic acid as a potent antiepileptic molecule, there is tremendous interest in the search for derivatives with improved pharmacokinetic or safety profiles for epilepsy condition, such as bipolar disorders and epilepsy (valnoctamide **2**),[1] diisopropyl acetamide **3** (PID), valrocemide (valproyl glycinamide **4**), *N*-2,2,3,3-pentamethylcyclopropane carboxamide **5**, 2,2,3,3-tetramethylcyclopropylcarbonyl urea **6a**, isovaleramide **6b** (NPS-1776), and arundic acid **7** (ONO-2506),[2,3] Fig. 1. Beside an affective epileptic drug, valproic acid inhibits angiogenesis both *in vitro* and *in vivo*,[4–8] but no attempt has been made to synthesize valproic acid derivatives with improved antiangiogenic potential.

Hydrazide-hydrazones derivatives have been reported with different pharmaceutical applications, where they were considered as antibacterial, antifugal, antimicrobial and anticonvulsant agents. Many of them showed analgesic and antiplatelet properties.[9–16] In addition, they were reported to elicit anticancer[17–24] and anti-human immunodeficiency virus (HIV) properties[25] and hence they gained an important place in medicinal chemistry.[26–33]

The aim of the study is designing series of hydrazide-Schiff bases with a wide spectrum of pharmaceutical applications, having the valproic acid unit at the N-terminal and different Schiff bases at the C-terminal; considering some factors responsible for such activity which are i) the presence of electron-rich aromatic moieties; ii) the presence of amide and hydrazido functionality, iii) the valproic acid drug moiety. All the prepared compounds were tested for antiangiogenic activity as well as anticancer activity in HepG2 cell.

Valproic acid **1** was treated with methanol in the presence of conc. H_2SO_4 to afford the ester derivative which was used directly in the next step for preparation of the valproic acid hydrazide **8**.[34–36] Compound **8** was condensed with substituted benzaldehyde and acetophenone in the presence of 2–3 drops

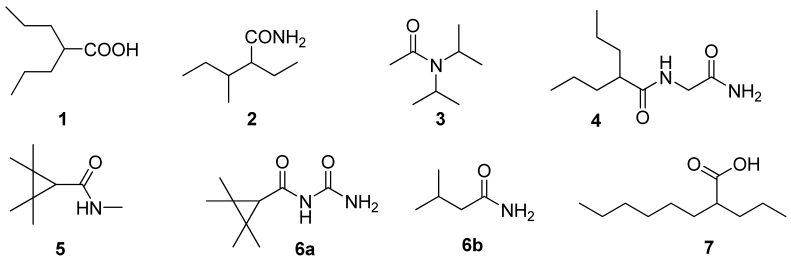

Fig. 1. Valproic Acid and Derivatives with Improved Pharmacokinetic or Safety Profiles for Epilepsy Condition

The authors declare no conflict of interest.

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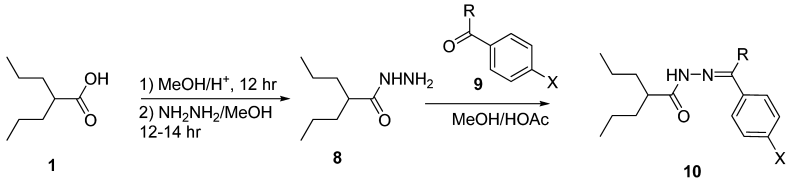

	Yiels %
R = X = H; **10a;**	87
R = H; X = Cl; **10b;**	87
R = H; X = OH; **10c;**	90
R = H; X = OCH_3; **10d;**	83
R = CH_3; X = H; **10e;**	91
R = CH_3; X = OH; **10f;**	86
R = CH_3; X = OCH_3; **10g;**	85

Chart 1. Synthesis of Schiff Bases of Valproic Acid Hydrazide

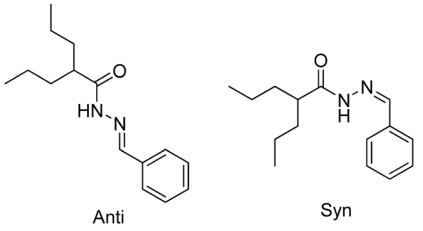

Fig. 2. The Expected Geometrical Isomers, *Anti* and *Syn* Forms, of Compound **10a**

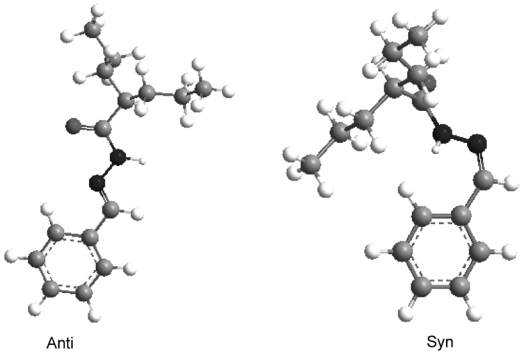

Fig. 3. Geometry Optimizations Using the DFT Level (B3LYP/6-31G**) of Theory for Schiff's Base **10a**, *Anti* and *Syn* Forms

of glacial HOAc in methanol as solvent to afford products **10a**–**g** (Chart 1). The structure of synthesized compounds **8** and **10a**–**g** was confirmed by spectral data.

The ^{1}H- and ^{13}C-NMR spectra of products **10a**–**g** indicate the presence of two isomeric forms. As a prototype, the ^{1}H-NMR spectrum of **10a** showed two singlet peaks equivalent to one proton at δ 8.20 and 7.97 ppm, in ratio 54.8%: 45.2%, corresponding to the sp^2 CH proton. In addition, two singlet peaks, which are D_2O exchangeable, were observed at 11.31 and 11.21 ppm corresponding to the NH proton. The ^{13}C-NMR spectrum showed two carbonyl signals at δ 172.24 and 177.86 ppm, which is a further confirmation of the presence of two isomers. It is expected that compound **10a** could adopt two different geometrical isomers as shown in Fig. 2. Therefore, it is considered worthwhile to carry out quantum chemical calculations with the GAUSSIAN 98 suite of programs. Geometry optimizations were carried out using the density functional theory (DFT) level (B3LYP/6-31G**) of theory to assess the relative stability of the *anti–syn* isomeric species. Calculated relative energies of **10a** *anti–syn* isomers are −768.8173146 au and −768.7634624 au, respectively. Computed energies indicate the stability of the anti isomer over the syn one by 0.0538522 au (33.79279 kcal/mol), Fig. 3.

The glycinate derivatives were synthesized by the reaction of the freshly prepared valproylchloride[37] with glycine methyl ester in the presence of triethylamine as a base. The valproylglycine ester obtained was treated with hydrazine hydrate in methanol to afford the hydrazide derivative **11** as white crystalline solid. The hydrazide **11** was condensed with substituted benzaldehyde and acetophenone to afford the products **12a**–**e** in good yield (Chart 2). The structures of the synthesized products **12a**–**e** were confirmed by spectral data.

The benzoate derivatives were synthesized by the reaction of the freshly prepared valproylchloride with ethyl 4-aminobenzoate hydrochloride **13** in the presence of triethyl amine as base at retention time (r.t.) in dichloromethane as solvent (Chart 3). Hydrazinolysis of **14** was carried out in methanol under reflux for 4 h; to afford the corresponding hydrazide **15** in 87% yield (Chart 3). The structure of compound **14** and **15** were confirmed by NMR spectra and was in agreement with the reported data in the literature.[38] The reaction of **15** with substituted aromatic aldehydes **16a**–**e** as well as with ketones **18a**–**e** in the presence of drops of glacial acetic acid afforded the corresponding Schiff bases **17a**–**e** and **19a**–**e** in good to excellent yields as shown in Chart 3. The structures of the synthesized products **17a**–**e** were confirmed by spectral data.

The synthesized Schiff bases of valproylglycine hydrazide in this study (**12a**–**e**, Chart 2) showed significant level of antiangiogenic activity in transgenic zebrafish embryos (Tg: *fli1*: enhanced green fluorescent protein (EGFP)) by blocking 70–80% of intersegmental (ISV) and 100% of subintestinal vein (SIV) blood vessels formation. The level of antiangiogenic activity varied with each compound. As shown in Table 1, compound **12a** was the most potent in term of inhibition of angiogenic blood vessels at minimum EC_{50} value.

Compopund **12a** suppressed 72% of intrasegmental and 100% of subintestinal blood vessels formation process at EC_{50} value of only 5 μM, followed by compound **12b** by blocking 72% of ISV and 100% of SIV with EC_{50} value of 7 μM. Compound **12c** inhibited more than 83% of ISV and 100% of SIV but with EC_{50} value of 10 μM. The ISV inhibition was not observed with compound **12d**; however, it blocked 100% of SIV at 3 dpf embryos. Compound **12e** failed to show any antiangiogenic activity in treated zebrafish embryos even using 10 fold more concentration (50 μM) as compared to **12a**.

Compounds **12a**–**e** were also screened for anticancer activ-

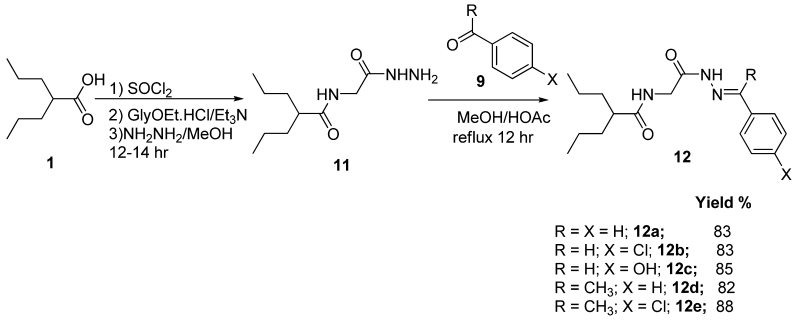

Chart 2. Synthesis of Schiff Bases of Valproylglycine Hydrazide

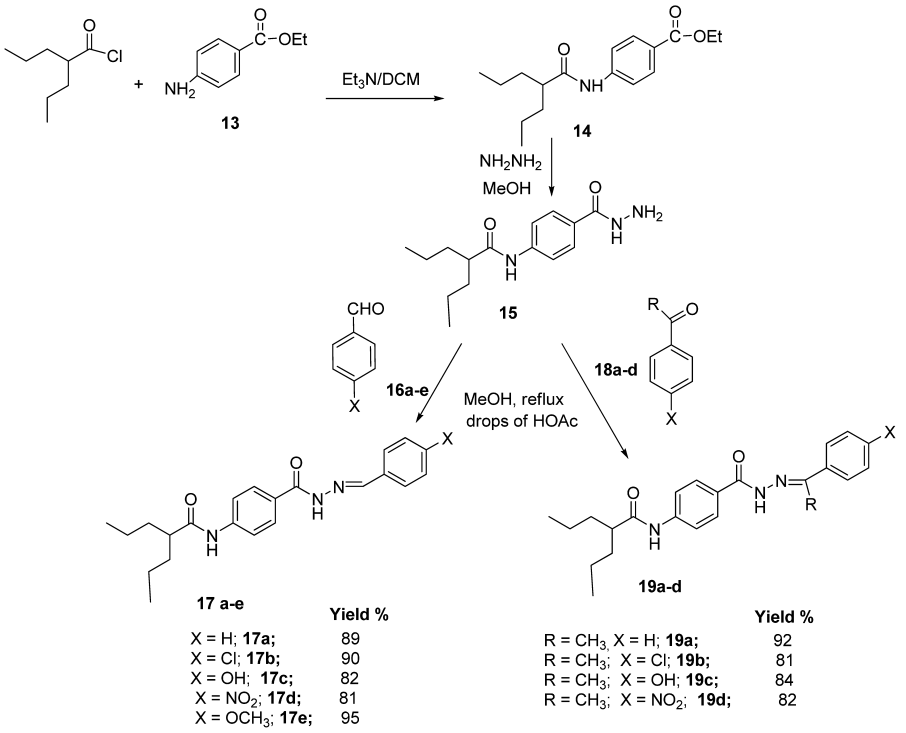

Chart 3. Synthesis of Schiff Bases of *N*-Valproyl-4-aminobenzoyl Hydrazide Derivatives

Table 1. Comparative Antiangiogenic and Anticancer Profile of Schiff Bases of Valporyglycine Hydrazide Derivatives and Valproic Acid

Compd.	Anti-angiogenic activity			Anticancer activity
	EC_{50} concentration (μM)	% ISV[a)] inhibition	% SIV[b)] inhibition	% Survival in HepG2 cells treated with 40 μM of comp.
12a	5	72±1.667[c)]	100±0[c)]	36.88±0.008[c)]
12b	7	72±0.666	100±0	71.34±0.009
12c	10	83±1.527	100±0.333	62.17±0.017
12d	32	0±0	100±0	78.62±0.009
12e	50	0±0	0±0	84.76±0.010
VPA	50	17±0.577	100±0	87.26±0.004
VALPH	80	0±0	0±0	83.47±0.010
Control	1% DMSO v/v	0±0	0±0	100.00 ±0.021

a) ISV: intersegmental vessels. *b*) SIV; subintestinal vein. *c*) Values are standard error of three different replicates.

ity in HepG2 liver cancer cell line. The cells were treated with 40 μM of compounds **12a**–**e** for 24 h and the effect on cell proliferation was checked by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As shown in Table 1, compound **12a** suppressed maximum level of cell growth inhibition of HepG2 cells by suppressing more than 60% of cell growth at 40 μM after 24 h of treatment followed by compound **12c** which suppressed 40% of cancer cell proliferation. Compound **12b** suppressed only 30% the cell growth. Compounds **12d** and **e** exhibited moderate level of anticancer activity at 40 μM by suppressing 20% and 15% of cell survival of HepG2 cancer cells at 40 μM. Valproic acid compound **1** could only block 13% of HepG2 cell proliferation at 40 μM, which means that the newly synthesized derivatives turnout to be more potent in term of anticancer activity and antiangiogenic activity as well. We have chosen the liver cancer cell line to evaluate the anticancer profile of valproic acid derivatives as valproic acid is finally metabolized in liver[39–41] and also the heptotoxicity is one of the major issue with valproic acid.[39,42–47]

Compared to Schiff bases of valproylglycine hydrazide **12a**–**e**, the Schiff bases of valproic acid hydrazide (**10a**–**g**) showed moderate level of antiangiogenic activity. As shown in Table 2, most of these compounds failed to affect the ISV blood vessels formation process in treated embryos, however they affected the SIV formation with EC_{50} value ranging from 40–50 μM. Compounds **10a**–**d** blocked 100% of subintestinal

Table 2. Comparative Antiangiogenic and Anticancer Profile of Schiff Bases of Valproic Acid Hydrazide Derivatives

Compd.	Anti-angiogenic activity in zebrafish embryos			Anticancer activity in HepG2 cells
	EC_{50} concentration (μM)	% ISV[a] inhibition	% SIV[b] inhibition	% Survival in HepG2 cells treated with 40 μM of comp.
10a	40	0±0[c]	100±0	57.58±0.025[c]
10b	45	0±0	100±0	68.30±0.044
10c	50	0±0	100±0	75.25±0.004
10d	50	0±0	100±0	72.96±0.009
10e	40	0±0	50±1.66	52.46±0.001
10f	80	0±0	0±0	75.85±0.007
10g	80	0±0	0±0	78.08±0.014

a) ISV: intersegmental vessels. *b*) SIV; subintestinal vein. *c*) Values are standard error of three different replicates.

Table 3. Comparative Antiangiogenic and Anticancer Profile of Schiff Bases of *N*-Valproyl-4-aminobenzoyl Hydrazide Derivatives

Compd.	Pro-angiogenic activity in zebrafish embryos		Anticancer activity in HepG2 cells
	EC_{50} concentration (μM)	# of blood vessels arcades in SIV[a] basket	% Survival in HepG2 cells treated with 40 μM of comp.
17a	100	13±0.33[b]	86.72±0.27[b]
17b	100	12±0.58	119.37±0.37
17c	100	12±0	79.45±0.25
17d	100	12±0.33	119.25±0.37
17e	100	12±0.33	112.48±0.35
19a	500	8±0	89.63±0.28
19b	500	8±0.33	119.30±0.37
19c	500	8±0	97.21±0.30
19d	500	8±0.33	115.46±0.36
1	50	8±0.33	122.78±0.38
15	40	18±0.33	95.78±0.30
14	40	15±0.33	92.80±0.29
Control 1% DMSO v/v		8±0.57	100.00±0.31

a) ISV: intersegmental vessels. *b*) Values are standard error of three different replicates.

vein, however compounds **10e**–**g** did not show significant level of antiangiogenic activity and only compound **10e** could inhibit 50% of SIV formation at 40 μM.

The anticancer activity of Schiff bases of valproic acid hydrazide are shown in Table 2. An inhibition of 25 to 40% of cell proliferation in HepG2 was observed with compounds **10a**–**g** at 40 μM.

The comparative angiogenic profile of valproyl-4-aminobenzoyl hydrazide derivatives **17a**–**e** and **19a**–**e** with **1**, revealed that in contrast to **1**, compounds **14**, **15** and **17a**–**e** promoted the angiogenic process in zebrafish embryos. These compounds modulated the subintestinal vein formation process by enhancing the number of blood vessels in SIV.

In Tg (*fli*-1: EGFP) transgenic zebrafish embryos SIV can easily be visualized at three days post fertilization as a smooth basket-like structure with 5–6 arcades. In zebrafish embryos treated with compounds **14**, **15** and **17a**–**e** and **19a**–**d**, a mesh of blood vessels with 15–18 arcades formed (Table 3). This pro-angiogenic activity was more prominent in embryos treated with 40 μM of either compound **14** or **15** at 3dpf. Schiff bases **19a**–**e** could not modulate the angiogenic process in zebrafish embryos even at very high concentration (500 μM). However, these compounds did not show any teratogenic or toxic affect at this very high concentration. One of the rational to design and synthesis new derivatives of **1**, is to overcome the severe toxicity attributed to it and to get effective epileptic compounds with minimum or no toxicity.

The Schiff bases of *N*-valproyl-4-aminobenzoyl hydrazide derivatives (**14**, **15**, **17a**–**e**, **19a**–**d**) showed a weaker level of anticancer activity *i.e.*, only 15–30% of suppressing the HepG2 cell survival (Table 3).

Conclusion

In conclusion, the synthesized compounds in this study modulated the angiogenic blood vessels formation in developing zebrafish embryos significantly. The Schiff bases of valproylglycine hydrazide exhibited strong antiangiogenic and anticancer activities, while on contrary Schiff bases of *N*-valproyl-4-aminobenzoyl hydrazide derivatives promoted the angiogenic process. Beside possessing a strong antiangiogenic activity these compounds did not show strong toxicity or teratogenecity in zebrafish embryos and specifically disrupted the angiogenic process, which mean that these compounds are targeted to those molecular and proteins involved in blood vessels formation process. The compounds have the potential to be promoted to pharmaceutical formulation as anticancer therapeutics or in condition which require the promotion of angiogenic blood vessels such as tissue regeneration process during wound healing.

Chemistry

General The solvents used were of HPLC reagent grade. Melting points were determined with a Mel-Temp apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 series Fourier transform instrument as KBr pellets. Nuclear magnetic resonance spectra (^{1}H- and ^{13}C-NMR spectra) were recorded on 400 MHz JEOL spectrometer at room temperature. Chemical shifts are reported in parts per million (ppm) and are referenced relative to residual solvent (*e.g.* $CHCl_3$ at δ_H 7.26 ppm for $CDCl_3$, dimethylsulfoxide (DMSO) at δ_H 2.50 ppm for DMSO-d_6). Spin multiplicities are represented by the following signals: singlet (s), broad singlet (brs), doublet (d), broad doublet (brd), doublet of doublets (dd), triplet (t), doublet of triplets (dt), quartet (q), sextet (sex) and multiplet (m). Elemental analyses were performed on Perkin-Elmer 2400 elemental analyzer, and the values found were within ±0.3% of the theoretical values. Follow-up of the reactions and checks of the purity of the compounds was done by TLC on silica gel-protected aluminum sheets (Type 60 GF254, Merck) and the spots were detected by exposure to UV-lamp at λ 254 nm for a few seconds. The compounds were named using Chem. Draw Ultra version 11, Cambridge Soft Corporation.

Synthesis of Valproic Hydrazide 8 Valproic acid (0.01 mol) was dissolved in 50 mL of methanol, and 3–4 drops of conc. sulphuric acid was added. The reaction mixture was refluxed for 12–14 h on water bath. The progress of the reaction was checked by TLC using hexane–ethyl acetate (4 : 6) as a mobile phase. After the reaction was completed, excess of methanol was removed under reduced pressure and the crude product was dissolved in ethyl acetate (30 mL), washed with

5% sodium bicarbonate solution (20 mL×2), water (20 mL×2), dried over anhydrous sodium sulphate, filtered, and then the solvent was removed under reduced pressure to afford the product as a viscous liquid in 90% yield.[34]

The crude product was used for the next step. Hydrazine hydrate (15 mL) was added to a solution of methyl valproate (0.01 mol) in methanol (20 mL) and the reaction mixture was refluxed for 12–14 h. The reaction mixture was cooled to r.t. to give the hydrazide **8** as a white colored shining fluffy product, in yield 85%, mp 123–124°C.[35] IR (KBr): 3284 (NH), 1631 (CO, amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.86 (t, 6H, $2CH_3$), 1.14–1.39 (m, 6H, $3CH_2$), 1.53–1.604 (m, 2H, CH_2), 1.95–2.01 (m, 1H, CH), 3.95 (br s, 2H, NH_2), 7.05 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.11, 20.86, 35.05, 45.61, 177.06.

General Method for Preparation of Schiff's Base of Valproic Hydrazide Derivatives 10a–g[36] A solution of valproic hydrazide **8** (0.2 g, 1.27 mmol) in methanol (25 mL) was added to a slotution of substituted benzaldehyde and acetophenone (1.27 mmol) in methanol (10 mL), and glacial acetic acid (2 drops), the reaction mixture was refluxed for 8 h. The product was separated out on cooling, filtered, recrystallized from ethanol to give 2-propylpentanehydrazide derivatives **10a**–**g**.

N-Benzylidene-2-propylpentanehydrazide (**10a**): The product was obtained as white crystals, in yield 0.27 g (87%), mp 105–106°C. IR (KBr): 3203 (NH), 1659 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): Isomer A (54.8%) δ (ppm): 0.82–0.86 (m, 6H, $2CH_3$), 1.19–1.36 (m, 6H, $3CH_2$), 1.48–1.56 (m, 2H, CH_2), 2.20–2.27 (m, 1H, CH), 7.38–7.41 (m, 3H, Ar-H), 7.60–7.66 (m, 2H, Ar-H), 8.20 (s, 1H, CH), 11.31 (s, 1H, NH). Isomer B (45.2%) δ (ppm): 0.82–0.86 (m, 6H, $2CH_3$), 1.19–1.36 (m, 6H, $3CH_2$), 1.48–1.56 (m, 2H, CH_2), 3.38–3.46 (m, 1H, CH), 7.38–7.41 (m, 3H, Ar-H), 7.60–7.66 (m, 2H, Ar-H), 7.97 (m, 1H, CH), 11.21 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.64, 20.80, 20.89, 34.94, 35.38, 44.77, 127.17, 127.61, 129.43, 129.53, 142.96, 146.78, 172.24, 177.86. *Anal.* Calcd for $C_{15}H_{22}N_2O$: C, 73.13; H, 9.00; N, 11.37. Found: C, 72.91; H, 8.79; N, 11.58.

N′-(4-Chlorobenzylidene)-2-propylpentanehydrazide (**10b**): The product was obtained as white crystals, in yield 0.31 g (87%), mp 155°C. IR (KBr): 3208 (NH), 1661 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): Isomer A (53.7%) δ (ppm): 0.82–0.84 (m, 6H, $2CH_3$), 1.20–1.55 (m, 8H, $4CH_2$), 2.21–2.26 (m, 1H, CH), 7.48–7.91 (m, 4H, Ar-H), 7.93 (s, 1H, CH), 11.38 (s, 1H, NH). Isomer B (46.3%) δ (ppm): 0.82–0.84 (m, 6H, $2CH_3$), 1.20–1.55 (m, 8H, $4CH_2$), 2.21–2.26 (m, 1H, CH), 7.48–7.91 (m, 4H, Ar-H), 8.19 (m, 1H, CH), 11.28 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.63, 14.65, 20.79, 20.88, 34.95, 35.36, 44.77, 129.39, 131.80, 134.05, 138.41, 141.74, 145.49, 167.13, 172.34. *Anal.* Calcd for $C_{15}H_{21}ClN_2O$: C, 64.16; H, 7.54; N, 9.98. Found: C, 63.94; H, 7.79; N, 9.78.

N′-(4-Hydroxybenzylidene)-2-propylpentanehydrazide (**10c**): The product was obtained as white crystals, in yield 0.30 g (90%), mp 190–192°C. IR (KBr): 3320–3200 (OH), 3218 (NH), 1632 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): Isomer A (60.5%) δ (ppm): 0.82–0.85 (m, 6H, $2CH_3$), 1.18–1.32 (m, 6H, $3CH_2$), 1.46–1.54 (m, 2H, CH_2), 2.17–2.22 (m, 1H, CH), 6.78 (d, 2H, J=8.4 Hz, Ar-H), 7.42–7.48 (m, 2H, Ar-H), 8.07 (s, 1H, CH), 9.82 (s, 1H, OH), 11.09 (s, 1H, NH). Isomer B (39.5%) δ (ppm): 0.82–0.85 (m, 6H, 2 CH_3), 1.18–1.32 (m, 6H, 3 CH_2), 1.46–1.54 (m, 2H, CH_2), 3.37–3.42 (m, 1H, CH), 6.78 (d, 2H, J=8.4 Hz, Ar-H), 7.42–7.48 (m, 2H, Ar-H), 7.86 (m, 1H, CH), 9.82 (s, 1H, OH), 10.99 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.65, 20.80, 20.89, 34.94, 35.43, 44.72. 116.30, 126.00, 126.13, 129.32, 143.24, 147.06, 159.88, 171.83, 177.47. *Anal.* Calcd for $C_{15}H_{22}N_2O_2$: C, 68.67; H, 8.45; N, 10.68. Found: C, 68.81; H, 8.55; N, 10.51.

N′-(4-Methoxybenzylidene)-2-propylpentanehydrazide (**10d**): The product was obtained as white crystals, in yield 0.29 g (83%), mp 110–112°C. IR (KBr): 3216 (NH), 1656 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): Isomer A (53.9%) δ (ppm): 0.82–0.86 (m, 6H, $2CH_3$), 1.18–1.33 (m, 6H, $3CH_2$), 1.47–1.54 (m, 2H, CH_2), 2.19–2.22 (m, 1H, CH), 3.78 (s, 3H,OCH_3), 6.96–6.98 (m, 2H, Ar-H), 7.54–7.60 (m, 2H, Ar-H), 8.13 (m, 1H, CH), 11.17 (s, 1H, NH). Isomer B (46.1%) δ (ppm): 0.82–0.86 (m, 6H, $2CH_3$), 1.18–1.33 (m, 6H, $3CH_2$), 1.47–1.54 (m, 2H, CH_2), 3.39–3.43 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 6.96–6.98 (m, 2H, Ar-H), 7.54–7.60 (m, 2H, Ar-H), 7.90 (m, 1H, CH), 11.07 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.65, 20.80, 20.89, 34.93, 35.41, 44.73, 55.95, 114.94, 129.18, 142.82, 142.84, 146.66, 171.96, 177.60. *Anal.* Calcd for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14. Found: C, 69.71; H, 8.54; N, 10.41.

N′-(1-Phenylethylidene)-2-propylpentanehydrazide (**10e**): The product was obtained as white crystals, in yield 0.30 g (91%), mp 118–119°C. IR (KBr): 3201 (NH), 1666 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): Isomer A (55.2%) δ (ppm): 0.81–0.87 (m, 6H, $2CH_3$), 1.20–1.35 (m, 6H, $3CH_2$), 1.48–1.60 (m, 2H, CH_2), 2.21 (s, 3H, CH_3), 2.50–2.51 (m, 1H, CH), 7.37–7.39 (m, 3H, Ar-H), 7.72–7.77 (m, 2H, Ar-H), 10.36 (s, 1H, NH). Isomer B (44.8%) δ (ppm): 0.81–0.87 (m, 6H, $2CH_3$), 1.20–1.35 (m, 6H, $3CH_2$), 1.48–1.60 (m, 2H, CH_2), 2.24 (s, 3H, CH_3), 3.48–3.52 (m, 1H, CH), 7.37–7.39 (m, 3H, Ar-H), 7.72–7.77 (m, 2H, Ar-H), 10.24 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.69, 14.74, 20.83, 20.87, 34.92, 35.49, 43.99, 126.46, 126.95, 128.92, 129.08, 147.22, 152.38, 172.72, 178.58. *Anal.* Calcd for $C_{16}H_{24}N_2O$: C, 73.81; H, 9.29; N, 10.76. Found: C, 73.61; H, 9.53; N, 10.48.

N′-(1-(4-Hydroxyphenyl)ethylidene)-2-propylpentanehydrazide (**10f**): The product was obtained as white crystals, in yield 0.30 g (86%), mp 183–184°C. IR (KBr): 3450–3200 (OH), 3281 (NH), 1660 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): Isomer A (48.9%) δ (ppm): 0.80–0.86 (m, 6H, $2CH_3$), 1.17–1.37 (m, 6H, $3CH_2$), 1.47–1.58 (m, 2H, CH_2), 2.14 (s, 3H, CH_3), 2.45–2.50 (m, 1H, CH), 6.74–6.83 (m, 2H, Ar-H), 7.56–7.62 (m, 2H, Ar-H), 9.68 (s, 1H, OH), 10.17 (s, 1H, NH). Isomer B (51.1%) δ (ppm): 0.80–0.86 (m, 6H, $2CH_3$), 1.17–1.37 (m, 6H, $3CH_2$), 1.47–1.58 (m, 2H, CH_2), 2.16 (s, 3H, CH_3), 3.43–3.52 (m, 1H, CH), 6.74–6.83 (m, 2H, Ar-H), 7.56–7.62 (m, 2H, Ar-H), 9.68 (s, 1H, OH), 10.10 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ (ppm): 14.71, 14.73, 20.83, 20.88, 34.94, 35.53, 44.03, 115.65, 115.79, 115.81, 127.95, 128.51, 159.27, 172.34, 178.28. *Anal.* Calcd for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14. Found: C, 69.69; H, 8.63; N, 10.38.

N′-(1-(4-Methoxyphenyl)ethylidene)-2-propylpentanehydrazide (**10g**): The product was obtained as white crystals, in yield 0.31 g (85%), mp 117–118°C. IR (KBr): 3232 (NH), 1649 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): Isomer A (47.4%) δ (ppm): 0.81–0.87 (m, 6H, $2CH_3$), 1.20–1.36 (m, 6H, $3CH_2$), 1.47–1.59 (m, 2H, CH_2), 2.18 (s, 3H, CH_3), 2.5–2.55 (m, 1H, CH), 3.75–3.77 (m, 3H, OCH_3), 6.93–6.95 (d, 2H, Ar-H), 7.67–7.72 (m, 2H, Ar-H), 10.24 (s, 1H, NH). Isomer B (52.6%) δ (ppm): 0.81–0.87 (m, 6H, $2CH_3$), 1.20–1.36 (m, 6H,

3CH_2), 1.47–1.59 (m, 2H, CH_2), 2.20 (s, 3H, CH_3), 3.45–3.52 (m, 1H, CH), 3.75–3.77 (m, 3H, OCH_3), 6.93–6.95 (d, 2H, Ar-H), 7.67–7.72 (m, 2H, Ar-H), 10.14 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.71, 14.74, 20.83, 20.88, 34.93, 35.52, 55.88, 114.28, 114.46, 127.88, 127.43, 172.45, 178.36. *Anal.* Calcd for $C_{17}H_{26}N_2O_2$: C, 70.31; H, 9.02; N, 9.65. Found: C, 70.60; H, 8.83; N, 9.48.

Synthesis of *N*-(2-Hydrazinyl-2-oxoethyl)-2-propylpentanamide 11 Ethyl glycinate hydrochloride (3.48 g, 25 mmol) was mixed with 2 eq triethylamine (7 mL, 50 mmol) in 200 mL dichloromethane, while stirring continuously at 0°C. Freshly distilled valproyl chloride (4.1 g, 25 mmol) dissolved in 50 mL of dichloromethane and added dropwise, after complete addition, the reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with water (2×20 mL), dried over $MgSO_4$ anhydrous, filtered and the solvent was removed under reduced pressure, the residue was recrystallized from acetone to afford ethyl 2-(2-propylpentanamido)acetate in yield 5.5 g (95%), mp 82°C.

To a solution of ethyl 2-(2-propylpentanamido)acetate (4.6 g, 20 mmol) in 10 mL methanol, 10 mL hydrazine hydrate (80%) was added. The reaction mixture was refluxed for about 4 h, and the excess of hydrazine was evaporated under reduced pressure. The product that precipitated was filtrated off and washed several time with ethanol. The product **11** was obtained as white crystals in yield 3.9 g (90.6%), mp 245–246°C. ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.77 (m, 6H, 2CH_3), 1.16 (m, 6H, 3CH_2), 1.37 (m, 2H, CH_2), 2.16 (m, 1H, CH), 3.65 (s, 2H, CH_2), 4.25 (brs, 2H, NH_2), 8.03 (m, 1H, NH), 9.84 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 8.99, 14.58, 20.62, 35.37, 35.42, 40.91, 45.53, 45.96, 169.09, 175.89.

General Procedure for Preparation of Schiff's Base of *N*-(2-Hydrazinyl-2-oxoethyl)-2-propylpentanamide Derivatives 12a–e A solution of *N*-(2-hydrazinyl-2-oxoethyl)-2-propylpentanamide **11** (0.22 g, 1 mmol) in methanol (25 mL) was added to substituted aldehydes or ketones (1 mmol) in methanol (10 mL), and glacial acetic acid (2 drops) and the reaction mixture was then refluxed for 8 h. The product was separated out on cooling, filtered off, recrystallized from ethanol and dried to give the *N*-(2-hydrazinyl-2-oxoethyl)-2-propylpentanamide derivatives **12a**–**e**.

N-(2-(2-Benzylidenehydrazinyl)-2-oxoethyl)-2-propylpentanamide (**12a**): The product was obtained as white crystals, in yield 0.25 g (83%), mp 80–81°C. ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.82 (t, 6H, 2CH_3), 1.19–1.34 (m, 6H, 3CH_2), 1.40–1.47 (m, 2H, CH_2), 2.11–2.23 (m, 1H, CH), 3.59 (s, 2H, CH_2), 7.58–7.68 (m, 4H, Ar-H+NH), 7.93–7.95 (d, 2H, Ar-H), 8.70 (s, 1H, CH), 11.22 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.57, 14.65, 14.68, 20.71, 20.81, 34.83, 35.56, 45.67, 62.20, 129.04, 129.58, 132.02, 134.48, 162.13. *Anal.* Calcd for $C_{17}H_{25}N_3O_2$: C, 67.30; H, 8.31; N, 13.85. Found: C, 67.57; H, 8.25; N, 13.78.

N-(2-(2-(4-Chlorobenzylidene)hydrazinyl)-2-oxoethyl)-2-propylpentanamide (**12b**): The product was obtained as white crystals, in yield 0.28 g (83%), mp 130–131°C. ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.79–0.83 (m, 6H, 2CH_3), 1.15–1.30 (m, 6H, 3CH_2), 1.39–1.44 (m, 2H, CH_2), 2.19–2.31 (m, 1H, CH), 4.19 (s, 2H, CH_2), 7.33 (d, 2H, Ar-H), 7.65–7.69 (m, 3H, Ar-H+NH), 8.70 (s, 1H, CH), 11.26 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.74, 20.77, 21.98, 35.60, 45.76, 127.29, 128.51, 128.58, 135.60, 136.03, 137.25, 168.91, 168.92. *Anal.* Calcd for $C_{17}H_{24}ClN_3O_2$: C, 60.44; H, 7.16; N, 12.44. Found: C, 60.17; H, 6.95; N, 12.68.

N-(2-(2-(4-Hydroxybenzylidene)hydrazinyl)-2-oxoethyl)-2-propylpentanamide (**12c**): The product was obtained as white crystals, in yield 0.27 g (85%), mp 253–254°C. ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.82 (t, 6H, 2CH_3), 1.19–1.34 (m, 6H, 3CH_2), 1.40–1.47 (m, 2H, CH_2), 2.11–2.23 (m, 1H, CH), 3.59 (s, 2H, CH_2), 7.58–7.68 (m, 3H, Ar-H+NH), 7.93–7.95 (d, 2H, Ar-H), 8.70 (s, 1H, CH), 11.33 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.57, 14.65, 14.68, 20.71, 20.81, 34.83, 35.56, 45.67, 62.20, 129.04, 129.58, 132.02, 134.48, 162.13. *Anal.* Calcd for $C_{17}H_{25}N_3O_3$: C, 63.93; H, 7.89; N, 13.16. Found: C, 64.21; H, 8.05; N, 13.44.

N-(2-Oxo-2-(2-(1-phenylethylidene)hydrazinyl)ethyl)-2-propylpentanamide (**12d**): The product was obtained as white crystals, in yield 0.26 g (82%), mp 105–106°C. ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.82–0.85 (m, 6H, 2CH_3), 1.23–1.26 (m, 6H, 3CH_2), 1.40–1.47 (m, 2H, CH_2), 2.15–2.23 (m, 1H, CH), 2.26 (s, 3H, CH_3), 4.25 (s, 2H, CH_2), 7.38–7.40 (m, 3H, Ar-H+NH), 7.75–7.78 (d, 2H, Ar-H), 7.98 (brs, 1H, NH), 11.17 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.60, 14.75, 15.40, 20.79, 20.83, 22.17, 34.92, 35.63, 45.33, 45.78, 127.15, 129.10, 130.43, 138.57, 157.98. *Anal.* Calcd for $C_{18}H_{27}N_3O_2$: C, 68.11; H, 8.57; N, 13.24. Found: C, 67.97; H, 8.35; N, 13.48.

N-(2-(2-(1-(4-Chlorophenyl)ethylidene)hydrazinyl)-2-oxoethyl)-2-propylpentanamide (**12e**): The product was obtained as white crystals, in yield 0.31 g (88%), mp 145–146°C. ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.81–0.84 (m, 6H, 2CH_3), 1.21–1.25 (m, 6H, 3CH_2), 1.43–1.46 (m, 2H, CH_2), 2.19–2.23 (m, 1H, CH), 2.25 (s, 3H, CH_3), 4.23 (s, 2H, CH_2), 7.44 (d, 2H, Ar-H), 7.78 (d, 2H, 2 Ar-H), 7.98 (s, 1H, NH), 11.22 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.75, 15.36, 20.78, 21.12, 35.62, 49.83, 129.00, 129.16, 135.28, 137.25, 157.61, 168.61. *Anal.* Calcd for $C_{18}H_{26}ClN_3O_2$: C, 61.44; H, 7.45; N, 11.94. Found: C, 61.67; H, 7.21; N, 12.17.

Synthesis of Ethyl 4-(2-Propylpentanamido)benzoate (VABE, 14)[37] Ethyl 4-aminobenzoate **13** (25 mmol) was mixed with triethylamine (25 mmol) in 200 mL dichloromethane. While a continuous stirring at 0°C; freshly distilled valproyl chloride (25 mmol) mixed with 50 mL dichloromethane was added dropwise and the reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL), the organic phase washed with water, and dried over $MgSO_4$ anhydrous. Filtered and the solvent was removed with a rotary evaporator to afford **14** in yield 6 g (82%) as an oily product. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.85 (t, 6H, J=7.3 Hz, 2 CH_3), 1.19–1.32 (m, 9 H, 3 CH_2, CH_3), 1.53–1.55 (m, 2H, CH_2), 2.44–2.50 (m, 1H, CH), 4.26 (q, 2H, J=7.3 Hz, CH_2), 7.77 (d, 2H, J=8.8 Hz, Ar-H), 7.90 (d, 2H, J=8.8 Hz, Ar-H), 10.25 (s, 1H, NH).

Synthesis of *N*-(4-(Hydrazinecarbonyl)phenyl)-2-propylpentanamide (VABH, 15) To a solution of ethyl 4-(2-propylpentanamido)benzoate **14** (5.82 g, 20 mmol) in 10 mL methanol, 10 mL hydrazine hydrate (80%) was added. The reaction mixture was refluxed for about 4 h then cooled to room temperature. The excess of hydrazine was evaporated under reduced pressure. The product that precipitated was filtrated off and washed several time by ethanol. The product was obtained as white crystal in yield 4.8 g (87%), mp 235–236°C. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 0.83 (t, 6H,

J=6.9 Hz, $2CH_3$), 1.17–1.35 (m, 6H, $3CH_2$), 1.48–1.55 (m, 2H, CH_2), 2.36–2.39 (m, 1H, CH), 4.42 (br s, 2H, NH_2), 7.63 (d, 2H, J=9.2 Hz, Ar-H), 7.72 (d, 2H, J=9.2 Hz, Ar-H), 9.60 (s, 1H, NH), 10.03 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 14.54, 20.77, 35.34, 46.78, 118.95, 128.17, 128.24, 142.19, 166.06, 175.18.

General Procedure for Preparation of Schiff's Base of *N*-(4-(Hydrazinecarbonyl)phenyl)-2-propylpentanamide Derivatives 17a–e and 19a–d A solution of *N*-(4-(hydrazinecarbonyl)phenyl)-2-propylpentanamide **15** (0.19 g, 0.7 mmol) in methanol (25 mL) was added to a solution of substituted benzaldehyde or acetophenone (0.7 mmol) in methanol (10 mL), and glacial acetic acid (2 drops). The reaction mixture was refluxed for 8 h. The product was separated out on cooling, filtered off, recrystallized from ethanol and dried to give the *N*-(4-(hydrazinecarbonyl)phenyl)-2-propylpentanamide derivatives **17a**–**e** and **19a**–**d**.

N-(4-(2-Benzylidenehydrazinecarbonyl)phenyl)-2-propylpentanamide (**17a**): The product was obtained as white crystals, in yield 0.23 g (89%), mp 251–252°C. IR (KBr): 3450, 3290 (NH), 1646 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.86 (t, 6H, J=7.2 Hz, $2CH_3$), 1.20–1.39 (m, 6H, $3CH_2$), 1.50–1.59 (m, 2H, CH_2), 2.38–2.47 (m, 1H, CH), 7.41–7.46 (m, 3H, Ar-H), 7.70–7.75 (m, 4H, Ar-H), 7.87 (d, 1H, J=8.4 Hz, Ar-H), 8.44 (s, 1H, CH), 10.12 (s, 1H, NH), 11.72 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 14.68, 20.92, 35.47, 46.96, 119.16, 127.69, 127.73, 129.18, 129.51, 129.90, 130.64, 130.65, 130.69, 135.11, 143.01, 148.02, 148.05, 163.14, 175.44. *Anal.* Calcd for $C_{22}H_{27}N_3O_2$: C, 72.30; H, 7.45; N, 11.50. Found: C, 72.02; H, 7.25; N, 11.78.

N-(4-(2-4-Chlorobenzylidenehydrazinecarbonyl)phenyl)-2-propylpentanamide (**17b**): The product was obtained as white crystals, in yield 0.26 g (90%), mp 265–266°C. IR (KBr): 3466, 3294 (NH), 1681 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.82–0.86 (m, 6H, $2CH_3$), 1.22–1.35 (m, 6H, $2CH_2$), 1.50–1.55 (m, 2H, CH_2), 2.35–2.42 (m, 1H, CH), 7.54 (d, 2H, J=8.8 Hz, Ar-H), 7.64 (d, 2H, J=8.8 Hz, Ar-H), 7.74 (d, 2H, J=8.8 Hz, Ar-H), 7.91 (d, 2H, J=8.8 Hz, Ar-H), 8.43 (s, 1H, CH), 9.60 (s, 1H, NH), 10.03 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 14.67, 20.90, 35.47, 46.93, 119.10, 119.15, 119.18, 128.31, 128.37, 129.39, 129.61, 131.80, 138.41, 142.33, 166.21, 167.15, 175.31. *Anal.* Calcd for $C_{21}H_{26}ClN_3O$: C, 67.82; H, 7.05; N, 11.30. Found: C, 68.04; H, 7.28; N, 11.58.

N-(4-(2-4-Hydroxybenzylidenehydrazinecarbonyl)phenyl)-2-propylpentanamide (**17c**): The product was obtained as white crystals, in yield 0.22 g (82%), mp 220–221°C. IR (KBr): 3300–3200 (OH), 3300, 3235 (NH), 1662 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.84 (t, 6H, J=7.2 Hz, $2CH_3$), 1.16–1.38 (m, 6H, $3CH_2$), 1.47–1.59 (m, 2H, CH_2), 2.37–2.44 (m, 1H, CH), 6.81 (d, 2H, J=8.4 Hz, Ar-H), 7.53 (d, 2H, J=8.4 Hz, Ar-H), 7.72 (d, 2H, J=8.8 Hz, Ar-H), 7.85 (d, 2H, J=8.8 Hz, Ar-H), 8.32 (s, 1H, CH), 9.98 (s, 1H, OH), 10.11 (s, 1H, NH), 11.51 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 14.67, 20.92, 35.47, 46.96, 116.37, 116.49, 119.15, 119.28, 119.30, 126.07, 129.06, 129.46, 142.83, 148.39, 160.01, 162.91, 175.43. *Anal.* Calcd for $C_{22}H_{27}N_3O_3$: C, 69.27; H, 7.13; N, 11.02. Found: C, 69.04; H, 7.38; N, 11.28.

N-(4-(2-4-Nitrobenzylidenehydrazinecarbonyl)phenyl)-2-propylpentanamide (**17d**): The product was obtained as pale yellow crystals, in yield 0.24 g (81%), mp 278–280°C. IR (KBr): 3296, 3219 (NH), 1649 (CO, amide), 1548 and 1348 (NO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.86 (t, 6H, J=7.2 Hz, $2CH_3$), 1.23–1.38 (m, 6H, $3CH_2$), 1.52–1.55 (m, 2H, CH_2), 2.41–2.43 (m, 1H, CH), 7.75 (d, 2H, J=8.8 Hz, Ar-H), 7.89 (d, 2H, J=8.8 Hz, Ar-H), 7.95–7.97 (m, 2H, Ar-H), 8.28 (d, 2H, J=8.8 Hz, Ar-H), 8.53 (s, 1H, CH), 10.14 (s, 1H, NH), 12.02 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 14.68, 20.92, 35.46, 46.97, 119.17, 119.25, 124.77, 128.59, 129.38, 129.40, 141.45, 145.47, 148.46, 175.49. *Anal.* Calcd for $C_{22}H_{26}N_4O_4$: C, 64.37; H, 6.38; N, 13.65. Found: C, 64.14; H, 6.30; N, 13.38.

N-(4-(2-4-Methoxybenzylidenehydrazinecarbonyl)-phenyl)-2-propylpentanamide (**17e**): The product was obtained as white crystals, in yield 0.27 g (95%), mp 259–260°C. IR (KBr): 3306, 3218 (NH), 1647 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.85 (t, 6H, J=7.2 Hz, $2CH_3$), 1.19–1.39 (m, 6H, $3CH_2$), 1.50–1.57 (m, 2H, CH_2), 2.40–2.42 (m, 1H, CH), 3.79 (s, 3H, OCH_3), 6.99 (d, 2H, J=8.8 Hz, Ar-H), 7.64 (d, 2H, J=8.4 Hz, Ar-H), 7.72 (d, 2H, J=8.8 Hz, Ar-H), 7.85 (d, 2H, J=8.4 Hz, Ar-H), 8.37 (s, 1H, CH), 10.11 (s, 1H, NH), 11.58 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 14.68, 20.92, 35.47, 46.96, 55.98, 115.01, 119.15, 127.66, 129.29, 142.88, 142.89, 147.92, 161.45, 175.42. *Anal.* Calcd for $C_{23}H_{29}N_3O_3$: C, 69.85; H, 7.39; N, 10.62. Found: C, 69.74; H, 7.60; N, 10.48.

N-(4-(2-(1-Phenylethylidene)hydrazinecarbonyl)phenyl)-2-propylpentanamide (**19a**): The product was obtained as white crystals, in yield 0.25 g (92%), mp 220–221°C. IR (KBr): 3438, 3296 (NH), 1650 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.86 (t, 6H, J=7.2 Hz, $2CH_3$), 1.20–1.38 (m, 6H, $3CH_2$), 1.50–1.59 (m, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.38–2.45 (m, 1H, CH), 7.40–7.41 (m, 3H, Ar-H), 7.72 (d, 2H, J=8.8 Hz, Ar-H), 7.80–7.85 (m, 4H, Ar-H), 10.10 (s, 1H, NH), 10.59 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 14.68, 20.92, 35.48, 46.97, 118.97, 118.98, 119.00, 119.02, 119.05, 127.03, 129.02, 138.86, 175.41. *Anal.* Calcd for $C_{23}H_{29}N_3O_2$: C, 72.79; H, 7.70; N, 11.07. Found: C, 73.00; H, 7.44; N, 11.32.

N-(4-(2-(1-(4-Chlorophenyl)ethylidene)hydrazinecarbonyl)-phenyl)-2-propylpentanamide (**19b**): The product was obtained as white crystals, in yield 0.24 g (81%), mp 265–266°C. IR (KBr): 3463, 3250 (NH), 1663 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.85 (t, 6H, J=7.2 Hz, $2CH_3$), 1.18–1.39 (m, 6H, $3CH_2$), 1.50–1.59 (m, 2H, CH_2), 2.33 (s, 3H, CH_3), 2.38–2.45 (m, 1H, CH), 7.47 (d, 2H, J=8.4 Hz, Ar-H), 7.71 (d, 2H, J=8.8 Hz, Ar-H), 7.82–7.84 (m, 4H, Ar-H), 10.10 (s, 1H, NH), 10.63 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 14.68, 20.92, 35.48, 46.97, 118.97, 118.99, 119.01, 119.03, 128.75, 128.78, 128.82, 129.07, 129.65, 137.69, 175. 41. *Anal.* Calcd for $C_{23}H_{28}ClN_3O_2$: C, 66.74; H, 6.82; N, 10.15. Found: C, 66.57; H, 6.58; N, 10.32.

N-(4-(2-(1-(4-Hydroxyphenyl)ethylidene)hydrazine-carbonyl)phenyl)-2-propylpentanamide (**19c**): The product was obtained as white crystals, in yield 0.24 g (84%), mp 264–265°C. IR (KBr): 3300–3200 (OH), 3278 (NH), 1657 (CO, amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.85 (t, 6H, J=7.2 Hz, $2CH_3$), 1.20–1.39 (m, 6H, $3CH_2$), 1.50–1.59 (m, 2H, CH_2), 2.27 (s, 3H, CH_3), 2.38–2.45 (m, 1H, CH), 6.78 (d, 2H, J=8.4 Hz, Ar-H), 7.67–7.72 (m, 4H, Ar-H), 7.82 (d, 2H, J=8.4 Hz, Ar-H), 9.75 (s, 1H, OH), 10.09 (s, 1H, NH), 10.47 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 14.68, 20.93, 35.48, 46.96, 115.75, 119.03, 128.67, 128.68, 129.63, 137.89, 138.01, 142.58, 142.46, 142.67, 142.69, 142.75, 142.93, 159.51,

159.52, 175.37. *Anal.* Calcd for $C_{23}H_{29}N_3O_3$: C, 69.85; H, 7.39; N, 10.62. Found: C, 69.59; H, 7.48; N, 10.39.

N-(4-(2-(1-(4-Nitrophenyl)ethylidene)hydrazinecarbonyl)-phenyl)-2-propylpentanamide (**19d**): The product was obtained as pale yellow crystals, in yield 0.25 g (82%), mp 283–284°C. IR (KBr): 3298, 3220 (NH), 1649 (CO, amide), 1548 and 1346 (NO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.86 (t, 6H, J=7.2 Hz, $2CH_3$), 1.23–1.38 (m, 6H, $3CH_2$), 1.52–1.55 (m, 2H, CH_2), 2.41–2.43 (m, 1H, CH), 2.50 (s, 3H, CH_3), 7.75 (d, 2H, J=8.8 Hz, Ar-H), 7.89 (d, 2H, J=8.8 Hz, Ar-H), 7.95–7.97 (m, 2H, Ar-H), 8.28 (d, 2H, J=8.8 Hz, Ar-H), 10.14 (s, 1H, NH), 12.02 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 14.68, 20.92, 35.46, 46.97, 119.17, 124.77, 124.83, 128.59, 129.34, 129.38, 129.40, 141.45, 148.46, 175.49. *Anal.* Calcd for $C_{23}H_{28}N_4O_4$: C, 65.08; H, 6.65; N, 13.20. Found: C, 65.36; H, 6.48; N, 13.49.

Biology

Animals Wild type (*AB*/*Tuebingen TAB-14*) and Tg (*fli1*: EGFP)[48] zebrafish were obtained from zebrafish international resource center (ZIRC University of Oregon, Oregon, U.S.A.) and maintained in our facility under recommended conditions. The embryos were obtained by natural pair wise mating and treated following local and international guide lines for the use of laboratory animals.

Treatment of Zebrafish Embryos with VPA and Its Derivatives Stock Solutions: VPA and newly synthesized derivatives were dissolved in water and molecular biology grade dimethyl sulfoxide (DMSO) Sigma-Aldrich Cat # D8418 respectively to make a stock concentration of 20 mg/mL. The calculated volume of the compounds was added directly to Embryo Medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$ & 0.33 mM $MgSO_4$) to obtain required working dilutions. The mock (0.5% DMSO, v/v) treated embryos served as control.

Animal Treatment: Synchronized AB wild type embryos were raised to shield stage: (*ca.* 6 h post fertilization). The embryos were staged according to Kimmel *et al.*[49] Any unfertilized or embryo that appeared developmentally delayed or otherwise abnormal were also excluded. Around fifty (50) embryos were placed in 35 mm Petri dishes; in 10 mL embryo medium containing desired quantity of compound. The embryos were incubated in refrigerated air incubator at 28.5°C overnight. On the following day any dead embryos either in control or treated groups were recorded or removed and embryos were raised in compounds free embryo medium subsequently up to five days post fertilization (5 dpf) with replacement of embryo medium every day.

Antiangiogenic Assay in Zebrafish Embryos We have scored the antiangiogenic activity of the compounds in the live transgenic zebrafish embryos by observing the reduction in the outgrowth of blood vessels at two time points in the same embryo. i) ISV at 48 h post fertilization and then ii) SIV out growth at 72 h post fertilization. Just before counting the blood vessels, the embryos were anesthetized using 0.003% tricane (ethyl 3-aminobenzoate methanesulfonate, Sigma-Aldrich cat #E10521) in embryo medium. A scoring method was used to assess the level of antiangiogenic activity of compounds by counting total number of blood vessels in the trunk area of treated embryos and any missing or un-developed blood vessels. The percentage was calculated by using following equation.

$$\%\ \text{antiangiogenic activity} = \frac{\text{number of missing blood vessels in the trunk}}{\text{total number of blood vessels}} \times 100$$

At least three biological replications were conducted with different clutches of embryos from different parents. A concentration which was not inducing gross tearatological effects and only affecting the blood vessels outgrowth in zebrafish embryos was taken into consideration. A Compound was scored as antiangiogenic when it blocked the angiognesis blood vessels at least 70% of the treated embryos in all three biological replicates.

Calculation of EC_{50} EC_{50} values were calculated by treating the embryos with serial dilution of the compound and a mean value which inhibited the blood vessels formation in 50% of treated embryos in at least three replicates was taken as EC_{50}.

Cell Culture and Proliferation Assay HepG2 derived from human liver cancer were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM: Life Technologies Cat #11995073) supplemented with 10% fetal bovine serum (FBS: Life Technologies Cat #16000044) in a humidified incubator with 5% CO_2 at 37°C. Around 2×10^3 cells were seeded in each well of a 96-well cell culture plate and were allowed to adhere and spread for 24 h. The compounds were added to a final concentration of 40 μM in triplicate, and the cells were cultured for another 24 h at 37°C with the compounds. The proliferation was determined in each experiment using MTT 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan colorimetric assay. Briefly, the treated or untreated cells were trypsenized, centrifuged and the resulting pellet was resuspended in 100 μL of DMEM serum free medium in each well of 96-well plates and incubated at 37°C for two hours. After incubation 20 μL of MTT solution (5 mg/mL in phosphate buffered saline (PBS): Sigma-Aldrich Cat #M2003) was added to each well and further incubated for 2 h. The plate was centrifuged at 40000 rpm for 10 min then the medium was removed from each well and isopropanol containing 0.04 M HCl was added to dissolve the formazan produced in the cells. The optical density of the formazan product in solution was measured with a microplate reader at 540 nm. The experiment was conducted in triplicate. Data were calculated as percent of cell viability by the following formula:

$$\%\ \text{cell viability} = \frac{\text{mean absorbance in test wells}}{\text{mean absorbance in control wells}} \times 100$$

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