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Teratogenic Profile of Valproic Acid and Newly Synthesized Derivatives in Zebrafish Embryos

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ABSTRACT

In this study, the teratogenic profiles of five structural analogues of valproic acid (VPA) have been assessed in zebrafish embryos. The VPA derivatives induced severe teratogenecity in treated zebrafish embryos as compared to VPA. Moreover, three of these VPA derivatives specifically induced an undulation of notochord, which was not observed in VPA treated embryos. Variation in the treatment timings has shown that these compounds had affected the notochord cells differentiation process. Thus a new class of VPA analogues, which have affected notochord development process in zebrafish embryos by modulating the notochord cells differentiation and expansion, has been reported in this paper. © 2012 Friends Science Publishers

Key Words: VPA derivatives; Undulated notochord; Zebrafish

Abbreviations: VPA, Valproic acid; V-AA, 4-(2-propylpentanamido) benzoic acid; V-ABA 2-(2-propylpentanamido) benzoic acid; V-APAA, 2-(4-(2-propylpentanamido) phenyl) acetic acid; V-ABE Ethyl 4-(2-propylpentanamido) benzoate; V-ABH, N-(4-(hydrazinecarbonyl) phenyl)-2-propylpentanamide; hpf, Hours post fertilization; dpf , days post fertilization

INTRODUCTION

Valproic acid (VPA), is primarily used for the management of generalized and absence seizures in children (Henry, 2003). More recently; antitumor activity, inhibition of angiogenesis and differentiation of leukemic cells have been reported for VPA and its derivatives (Michaelis *et al.*, 2004; Deubzer *et al.*, 2006). VPA inhibits proliferation and induces neuronal differentiation of adult hippocampal neural progenitor cells in vitro and in vivo (Hsieh *et al.*, 2004; Yu *et al.*, 2009). Besides being very effective therapeutic agent in many diseases, concerns over its side effects mostly teratogenicity and hepatotoxicity have encouraged the need to develop structure analogues of VPA devoid of adverse effects (Jager-Roman *et al.*, 1986; Nau & Loscher, 1986; Haj-Yehia & Bialer, 1989; Nau *et al.*, 1991; Bojic *et al.*, 1996; 1998; Radatz *et al.*, 1998; Lu *et al*., 2004; Tasso *et al.*, 2004; Gravemann *et al.*, 2008).

Zebrafish are vertebrate organism that are of growing interest to be used for preclinical drug discovery applications. It has been extensively used as teratological and neuro-toxicological model (Van *et al.*, 1990; Powers *et al*., 2010). The toxicity of VPA derivatives synthesized in this study was tested in zebrafish embryos. All the compounds induced teratogenic phenotypes in zebrafish embryos like reduced pigmentation, cardiac edema, delayed hatching etc at concentration between 20 to 30 μM. However, the most prominent phenotype was formation of an undulated notochord. The variations in treatment timings suggested that these compounds could have affected the notochord development process specifically at the time of notochord cells differentiation in zebrafish embryos. Surprisingly, the parental molecule, (VPA) from which these compounds were derived, was not able to affect the notochord development process in zebrafish embryos.

MATERIALS AND METHODS

VPA and derivatives: VPA (2-Propylpentanoic acid, P6273), VPA sodium salt (P4543), Dimethyl sulfoxide DMSO (D8418 cell culture grade) was purchased from Sigma LLC. - ST. LOUIS, USA.

Animals: Wild type zebrafish (*AB/Tuebingen TAB-14*) were obtained from zebrafish international resource center (ZIRC University of Oregon, Oregon, USA) and maintained in Department of Zoology, King Saud University, Saudi

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Arabia. The embryos were obtained by natural pair wise mating. All experiments were carried out in accordance with National and International animal use guide lines and approved by the Institutional Animal Care and Use Committee at the King Saud University.

Treatment of Zebrafish Embryos with VPA and its Derivatives

Stock solutions: VPA was dissolved in distilled water while, VPA derivatives (synthesized in this study) were dissolved in Dimethyl sulfoxide (DMSO) to make a stock concentration of 50 mM. (VPA is un-soluble in DMSO but soluble in water whereas the VPA derivatives synthesized in this study are not soluble in water, therefore, two different solvent were used. All compounds were used in working dilution dissolved in DMSO 0.5% (v/v) in Embryo Medium (5 mM $NaCl_2$, 0.17 mM KCl_2, 0.33 mM $CaCl_2$ & 0.33 mM $MgSO_4$). The mock (1% DMSO) treated embryos served as control.

Animal treatment: Synchronized AB wild type embryos were raised to shield stage: (6 h post fertilization). For treatment the embryos were basically soaked with the drug solution prepared in embryo medium. Around fifty (50) embryos were placed in 35 mm Petri dishes; in 10 mL embryo medium containing compound or only DMSO. The embryos were incubated in refrigerated air incubator at 28.5°C overnight. The dead embryos in control or treated groups were recorded and removed and embryos were raised in compounds free embryo medium subsequently up to five days post fertilization (5 dpf) with replacement of embryo medium daily.

Microscopy and photography: Images were acquired using a Nikon Eclipse E600 Binocular Microscope, fitted with Nikon Digital Camera model DXM1200F, Japan.

Histology: The treated and mock treated embryos were euthanized by putting them on ice for 10 min in Petri dishes. The euthanized embryos were then fixed in 10% neutral buffered formalin (NBF) for 24 h at 4°C followed by rinsing with 70% ethanol, dehydrated in serial dilutions of ethanol before embedding in paraffin wax. Paraffin blocks of the tissues were sectioned at 5 μm thickness in a rotary microtome. Sections were processed for staining with haematoxyllin and eosin for histopathological details. Photographs of the sections were taken at different magnifications using a Nikon Eclipse E600 Binocular Microscope, fitted with Nikon Digital Camera model DXM1200F, Japan.

RESULTS

1- VPA derivatives as inducer of toxicity and teratgenicity in treated zebrafish embryos: In this study, five compounds are synthesized which are structural analogous of VPA. The structures of compounds are represented in Fig. 1. Zebrafish embryos were treated with serial dilution of these derivatives in order to evaluate the toxicity of these compounds. The compounds were added to embryos at 50% epiboly stage (6 h post fertilization). As shown in Table I all compounds were lethal to zebrafish embryos at concentrations above than 50 μM and had killed all the developing embryos within 18 h of treatment, except V-AA and V-APAA where the 100% mortality was observed at or more than 90 μM. whereas the VPA up to 50 μM was well tolerated by the embryos (Table I). The mock 1% DMSO (v/v) treated embryos survived and developed very well.

The zebrafish embryos provide very quick assessment of the teratogenic profile of the compounds. The embryos are treated with serial dilution of the compounds for specified time point (usually up to 24 hpf). A dose could be chosen, which does not induce severe mortality but produce some localized defect in developing organ of the embryos. The abnormalities in embryonic development for example pigmentation defects, shortened body axis, cardiac edema, bending of tail, slow or absence of circulation were collectively recorded as teratogenic affects. In order to get the comparative teratogenic profile of these compounds, a dose was fixed for all the compounds at which minimum mortality was observed but induced somewhat localized defects in developing zebrafish embryos. As depicted in Table II, VPA derivatives turned out to be more teratogenic as compared to VPA at 30 μM.

2- VPA derivatives induced notochord defects in the developing zebrafish embryos at sub lethal dose: Three VPA derivatives among five disrupted the notochord development in developing zebrafish embryos. The treated embryos had either undulated or bulging of notochord in trunk region. The embryos exposed to 4-(2-propylpentanamido) benzoic acid (20–40 μM) had an undulated notochord in the trunk region (Table III; Fig. 2 C-D-K-L). This undulation seems to be due to the disorganization of sheath cells in notochord throughout the whole trunk. The notochords in untreated or mock (DMSO 1% v/v) treated embryos developed normally and were straight having well organized notochord cells (Fig. 2, A, B, I–J). The second compound, 4-(2-propylpentanamido) benzoic acid, induced similar notochord defects at 20–40 μM in 70% of the treated embryos (Table II; Fig. 2, E, F, M–N). The zebrafish embryos that were treated with 2-(4-(2-propylpentanamido) phenyl) acetic acid did not induced the undulation in notochord, rather, 50% of embryos had bulging of notochord in the trunk region (Table II; Fig. 2G, H, O, P). The notochord abnormalities, which were induced by these compounds were irreversible as the treated embryos could not be rescued even growing them without the compounds up to 5 dpf (Fig. 2, K–P).

The two remaining VPA derivatives synthesized in this study i.e., Ethyl 4-(2-propylpentanamido) benzoate and (4-(hydrazinecarbonyl) phenyl)-2-propylpentanamide induced somewhat totally different phenotype in treated zebrafish embryos. These two compounds were most toxic

Table I: Dose response of zebrafish embryos to mortality on exposure to various concentrating of VPA derivatives and VPA

Concentration of Compound in µM	Total number of embryos used**	% Mortality**						
		V-AA	V-APAA	V-ABA	V-ABH	V-ABE	VPA	Control 1% DMSO (v/v) *
	50	-	-	-	-	-	-	1.66
10	50	3.33	1.33	3.33	3.33	4.66	1.33	
20	50	3.33	3.33	6.66	10	13.33	3.33	
30	50	4.66	6.66	33.33	46.66	66.66	4.66	
40	50	6.66	16.66	46.66	90	80	6.66	
50	50	13.33	66.66	66.66	80	100	6.66	
60	50	46.67	80	100	100	100	13.33	
90	50	93.33	100	100	100	100	33.33	
100	50	100	100	100	100	100	33.33	

*****All the compounds are in µM concentration except DMSO which is in % age

******The values are mean of three different treatments

Abbreviation used: VPA, Valproic acid; V-AA, 4-(2-propylpentanamido) benzoic acid; V-ABA, 2-(2-propylpentanamido) benzoic acid; V-APAA, 2-(4-(2-propylpentanamido) phenyl) acetic acid; V-ABE, Ethyl, 4-(2-propylpentanamido) benzoate; V-ABH, N-(4-(hydrazinecarbonyl) phenyl)-2-propylpentanamide

Table II: Comparative teratogenic profile of valproic acid and synthesized structural analogues in zebrafish embryos treated at 30 µM

Total number of embryos used **		% Comparative teratogenic profile at 30 µM **						
		V-AA	V-APAA	V-ABA	V-ABH	V-ABE	VPA	Control 1% DMSO (v/v) *
50	Reduced pigmentation	1.33	1.33	2	100	100	1	0.33
50	Cardiac edema	None	None	1	100	100	100	None
50	Bending of tail	None	None	None	80	100	None	None
50	Slow or absence of circulation	50	50	30	100	100	None	None
50	Un hatched	100	100	100	100	100	None	None

*All the concentration in micromoler except DMSO which is in percentage (v/v)

******The number is mean value of at least three different experiments

Table III: Dose response of zebrafish embryos to VPA and its derivatives in term of notochord abnormalities

Name of compounds	Number of embryos used	Embryos with notochord defects* Concentration of compounds				
	#	0.5% DMSO	10 µM	20 µM	30 µM	40 µM
Control DMSO **$** (0.5%)	50	0				
V-AA	50		0	35	45	45
V-ABA	50		0	25	30	40
V-APAA	50		0	20	25	30
V-ABH	50		0	0	0	0
V-ABE	50		0	0	0	0
VPA	50		0	0	0	0

* # The values are Average from triplicate experiment

$ The concentration of all the compounds are in µM except DMSO which is in percentage

Abbreviation used: VPA, Valproic acid; V-AA, 4-(2-propylpentanamido) benzoic acid; V-ABA, 2-(2-propylpentanamido) benzoic acid; V-APAA, 2-(4-(2-propylpentanamido) phenyl) acetic acid; V-ABE Ethyl, 4-(2-propylpentanamido) benzoate; V-ABH, N-(4-(hydrazinecarbonyl) phenyl)-2-propylpentanamide

Table IV: Response of zebrafish embryos to V-AA treatment for specified timings on notochord formation

	Numbers of embryos treated	Timings of treatment			Effected notochord?
		Compounds Added (Developmental stage)	Compounds Removed (Developmental stage)	Total time of treatment	
1	#200 (n*=3)	50% epiboly	5 prim (24 hpf)	18 hours	YES
2	150 (n*=3)	One cell	Sphere (4 hpf)	4 hours	NO
3	150 (n*=3)	5 prim (24 hpf)	Long Pec fin (48 hpf)	24 hours	NO

n*= Number of replication

The values are

and 80-100% of treated embryos died on second day exposed only to 40-µM dose. These two compounds also induced severe teratogenic phenotypes at lower concentration (10-30 µM Surprisingly the notochord development in Ethyl 4-(2-propylpentanamido) benzoate and (4-(hydrazinecarbonyl) phenyl)-2-propylpentanamide treated embryos was normal. As shown in Fig. 3C, E, D, F the treated embryos had normal i.e., straight notochords even though having very severe teratogenic phenotype.

3- The notochord abnormalities in zebrafish embryos were attributed specifically to VPA derivatives not VPA: VPA has been shown to induce multiple developmental

Fig. 1: Chemical structures of VPA derivatives

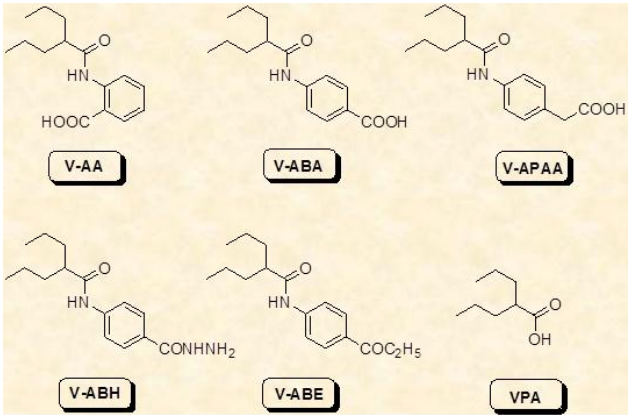
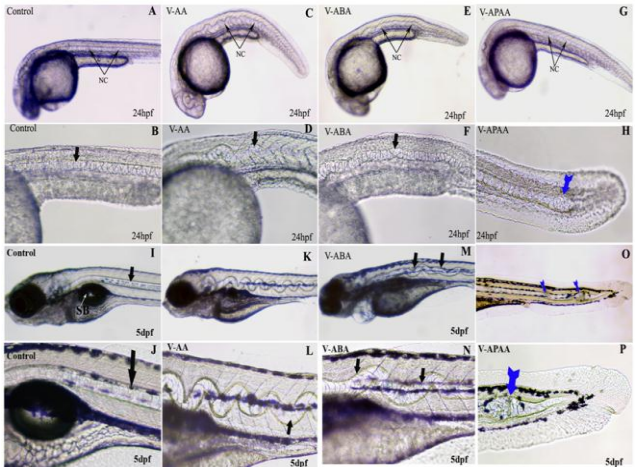


Fig. 2: VPA derivatives (V-AA, V-ABA & V-APAA) induced undulated or bulged notochord in zebrafish embryos

Zebrafish embryos were treated with VPA derivatives at 50 % epiboly stage. VPA derivatives induced abnormalities especially in developing notochords of the treated embryos. The control notochord is straight at 24 hpf (arrows in A, and arrow head in B) while the notochord has undulated phenotype in the V-AA treated (C, D, K L), V-ABA treated (E, F, M, The V-APAA treated embryos have bulging of notochord at posterior region of trunk represented by solid blue arrows (H, O, P). Top panel (A, C, E, G) are images from 24hpf embryos while second panel (B, D, F, H) are images at higher magnification from the top panel. The third panel (I,K,M,O) are images from 5dpf embryos and lowest panel (J,L,N,P) are in higher magnification of the images above panel



defects like shortened body axis, cardiac edema, reduced pigmentation, hepatotoxicity and angiogenesis blood vessels formation defects in wild type as well as transgenic zebrafish embryos (Mayerhoff *et al.*, 2005; Farooq *et al.*, 2008). However, there was no report prior to this study about VPA as inducer of notochord abnormalities in zebrafish or any other model organism. In order to evaluate the role of VPA in zebrafish notochord formation, the wild type zebrafish embryos were treated with various concentrations of VPA (20 to 40 µM from 50% epiboly stage for 18 h. As shown in Fig. 4, C-D and Table III the formation or development of notochord in VPA treated

Fig. 3: V-ABH and V-ABE had induced gross abnormalities but not malformed notochord in treated zebrafish embryos

Treating the embryos with VPA derivative V-ABH (C, D) and V-ABE (E, F) at 50 µM induced severe teratogenic effect. The embryos at 4dpf had severe cardiac edema (black arrow head), smaller head and the heart was malformed. They were developmentally delayed compared to control (A, B). The notochord in the treated embryos did not shown any obvious abnormalities (black arrow C-F)

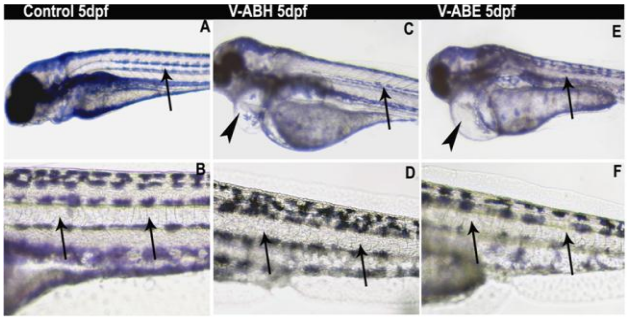
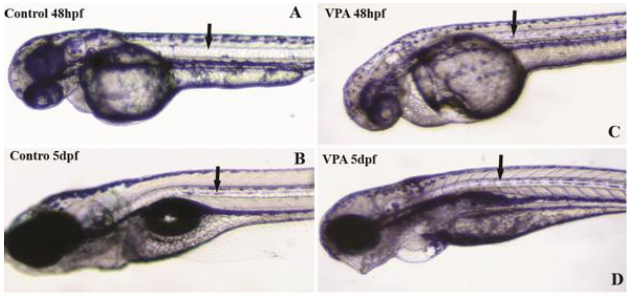


Fig. 4: Valproic acid did not induce notochord abnormalities in zebrafish embryos

The comparative notochord abnormalities between valproic acid and its derivatives showed that the notochord formation in Valproic acid treated embryos was normal. Zebrafish embryos were treated with VPA (30µM) from 50 % epiboly stage up to 18 hours, and subsequently grown without VPA from 24 hpf onward. There were no obvious abnormalities observed in the notochords of Valproic acid treated embryos (black arrow). The notochords were straight and cells were organized at 48 hpf (C) and at 5 dpf (D) as well



embryos were not affected. This mean that notochord abnormalities observed in treated embryos were attributed only to structure analogues of VPA synthesized in this study not the VPA itself.

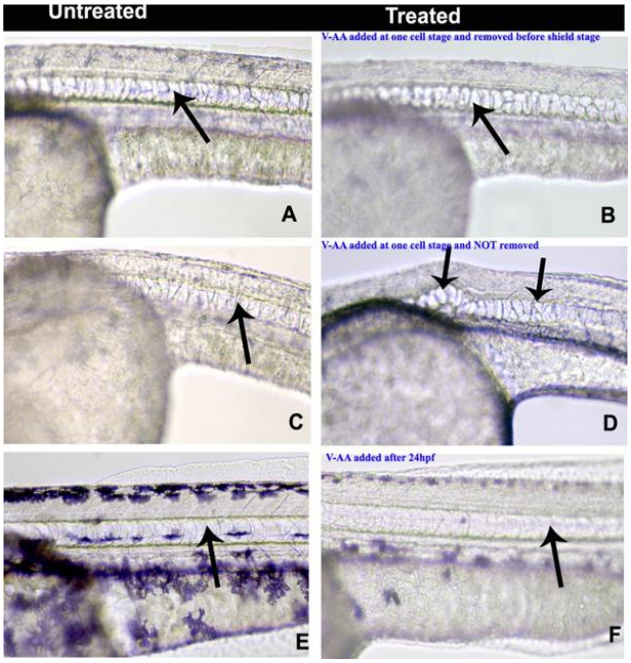
4- 2-(2-propylpentanamido) benzoic acid (V-AA) affected the notochord cells differentiation process in zebrafish treated embryos: The notochord cells in zebrafish are specified during gastrulation around 6 hp. The process begins by aligning of notochord cells in midline in the trunk (specification), and then expansion and straightening (differentiation & growth) by 24 hpf; (Odenthal *et al.*, 1996; Stemple *et al.*, 1996; Glickman *et al.*, 2003). That means this is the critical time or time window (from 6 hpf – 24 hpf) for any chemical modifier to disrupt notochord development process in zebrafish embryos. The zebrafish embryos were treated within in this critical time window in order to check that which process of notochord development i.e., specification, differentiation or growth was affected by these compounds? The zebrafish embryos

were exposed to V-AA for specified timings starting from as early as one cell stage up to 24 hpf stage. The undulation of notochord in V-AA treated zebrafish embryos was only observed when the embryos were exposed to V-AA between during 6 – 24 hpf (Table IV; Fig. 5D). The V-AA could not induce any abnormality in notochords, when zebrafish embryos were exposed with only for 5 h and was removed before 6 hpf (Fig. 5B) or embryos were treated after 24 hpf (Fig. 5F). That mean the V-AA had induced the undulation of notochord when embryos were exposed with within the critical time window of notochord cells formation process.

5- VPA derivatives treatment altered not only morphology but also the number of notochord cells in zebrafish embryos: In order to examine whether VPA derivatives treatment modulated the notochord cell morphology or proliferation, treated embryos were fixed at 5 days post fertilization and processed for histology. The undulation of the notochord in treated embryos made it very difficult to count the notochord cells in whole trunk and in some regions the cells are more deeply embedded in the trunk. Therefore, an unbiased criterion was set to count the cells in treated and untreated groups. We have counted the notochord cells, which were lying between three somites and an average was taken by counting the cells from at least three embryos from each group. H and E stained section clearly showed that the size and number of the cells was affected in treated embryos (Fig. 6B-D). In control embryos (Fig. 6A), the numbers of notochord cells were 9 under three somites, while these were in 21 in V-AA treated embryos, 18 in V-ABA and 40 in V-APAA. Moreover the morphology specially the size of the notochord cells was also altered in V-AA, V-ABA and V-APAA treated embryos. As shown in Fig. 6 black arrows in B,C,D the size of the notochord cells are much smaller compared to mock treated embryos (Fig. 6 A black arrow).

Fig. 5: V-AA treatment has specifically affected the differentiation of notochord cells in zebrafish embryos

A time window treatment of V-AA has shown that V-AA has affected the notochord at the time of notochord cells differentiation. V-AA was added and removed to zebrafish embryos for specified timings as mentioned in text. The left side panel shows the untreated embryoes and right side panel are the embryos treated with V-AA at different times. As shown in B, the V-AA was added at one cell stage and removed before the gastrulation stage. The notochord development was normal (black arrow), while the notochord was abnormal in those embryos which were continuously treated with V-AA for 18 hours (V-AA was not removed, D, black arrow). The notochord formation was also normal when V-AA was added to the embryos at later stage (24 hpf, F black arrow). The images from A-D are from 24 hpf and E - F from 48 hpf embryos



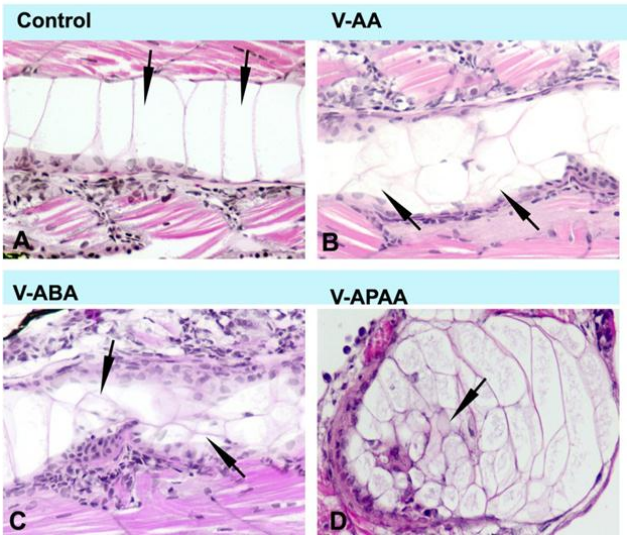
DISCUSSION

The antiepileptic drug VPA is increasingly used and investigated for pathological conditions, such as bipolar disorders, schizophrenia, migraine, and different pathologies of the brain (Loscher, 2002). Moreover, VPA is currently under clinical investigation as an anticancer drug for the treatment of gliomas in children (Blaheta *et al.*, 2002). The small molecular size and diverse biological activities of VPA attracted synthetic chemist to synthesize VPA derivatives (Jager-Roman *et al.*, 1986; Haj-Yehia & Bialer, 1989; Blaheta *et al.*, 2002; Loscher, 2002; Lu *et al.*, 2004; Tasso *et al.*, 2004). Moreover, most of these derivatives are the results of efforts to have a compound in hand, which has the same biological activity like VPA but less teratogenic. VPA is also a well-known modulator of angiogenesis *in vitro* and *in vivo* inhibiting angiogenesis both in developmental and pathological conditions (Michaelis *et al.*, 2004; Zgouras *et al.*, 2004; Isenberg *et al.*, 2007; Farooq *et al.*, 2008; Osuka *et al.*, 2012). We have modulated the basic structure of VPA to exploit its anti angiogenic potential in order to get potent anti-angiogenic compounds. The comparative anti-angiogenic profile of these compounds with VPA will be reported elsewhere but here we have reported the toxicity of these compounds using zebrafish as model organism. We have chosen zebrafish embryos to assess the toxicity and potential teratogenecity of these compounds as zebrafish embryos got many advantages over various model organism used for the purpose. The availability zebrafish embryos is frequent and in large in numbers. A single pair of adult zebrafish can produce 100-150 fertilized embryos each week. The embryos can easily be treated just soaking the embryos with the compounds and the bio availability of compound is quite higher compare to oral administration in mouse model or any other model. Moreover, the embryos can be treated in much less volume by treating them in 96 well micro titer plates, which provide much advantage when the compounds in use are available in minute quantities.

Three of these compounds (V-AA, V-ABA & V-APAA) induced abnormalities in the developing notochord,

Fig. 6: VPA derivatives disrupted the organization of notochord cells in embryos

Zebrafish embryos treated with V-AA (B) V-ABA (C) and V-APAA (D) or untreated (A) were grown up to 5 dpf, fixed and processed for haematoxylin and eosin (H&E) staining. The notochord cells are elongated and well organized in control embryos (A) whereas the VPA derivatives treated embryos not only had unorganized cells (black arrow in B C and D) Moreover, there were many smaller cells as well (black arrows). The V-APAA treated embryos had bulging of notochord cells in the trunk. The accumulation of lot of notochord cells can be easily visualized in this bulge (D)



which was wavy and bulged. Surprisingly this undulated notochord phenotype was not observed in VPA alone treated embryos, which mean that this phenotype was only due to the moieties, which were attached to the basic structure of VPA. The notochord abnormalities which were observed with these compounds have never been reported before with zebrafish or any other animal. That mean these compounds are quite novel in their bioactivity which need further to be explored.

The developmental process of notochord in zebrafish embryos starts around 6 hpf, when notochord cells are specified during gastrulation, then these specified cells come to lie in the midline, and expand and straighten in the trunk by 24 hpf (Odenthal *et al.*, 1996; Stemple *et al.*, 1996; Glickman *et al.*, 2003). Hence, a narrow temporal window between 6 to 24 hpf is very critical during which the notochord cells specify and differentiate and notochord cells were shown to be vulnerable to any chemical modifiers during this short temporal window (Anderson *et al.*, 2007). We also have used this short critical time window to observe the specific developmental process affected by VPA derivatives in zebrafish embryos. The notochord cells specified normally in VPA derivatives treated embryo as notochord was present at 24 hpf in treated embryos even though it was malformed and had undulation or bulging, that mean the first stage of notochord formation that is specification was not disrupted. However, the undulation or bulging of notochord cells in the trunk region means that aligning of the notochord cells in trunk was severely affected and the notochord cells in treated embryos were unable to align properly in trunk region under the influence of these compounds. The growth of the notochord cells was also affected in treated embryos as treated embryos had not only much less number of cells but these cells were of smaller size compared to control.

The undulation of notochord phenotype induced by VPA derivatives treatment was very much similar like fipronil (Stehr *et al.*, 2006), or 2-mercaptopyridine-*N*-oxide treated embryos (Anderson *et al*., 2007). The notochord formation and notochord cells differentiation was normal in zebrafish embryos exposed to 0.7 mM fipronil but later on displayed notochord degeneration, (Stehr *et al.*, 2006). As it has been mentioned above the VPA derivatives affected the notochord differentiation and growth process, once the notochord was formed in treated embryos it was not degenerated as in case of fipronil treatment. (Anderson *et al.*, 2007) has reported similar phenotype by exposing zebrafish embryos to 2-mercaptopyridine-*N*-oxide. Interestingly, 2-mercaptopyridine-*N*-oxide was much tolerated by zebrafish embryos and embryos survived even exposed to 100 µM 2-mercaptopyridine-*N*-oxide, whereas most of the VPA derivatives were lethal at this concentration (Table I).

The gross teratogenecity (absence of melanisation & shorted body axes) was very severe in zebrafish embryos treated with 2-mercaptopyridine-*N*-oxide even at very low dose (5 µM), while VPA derivatives did not produced any noticeable abnormalities even at 10 µM concentrations, that mean VPA derivatives are less teratogenic compared to 2-mercaptopyridine-*N*-oxide but more toxic. The zebrafish embryos treated with VPA derivatives were unable to recover even after growing them in compounds free medium for several days, which mean that these compounds had irreversibly affected signaling pathways, which are indispensible for notochord formation and organization of the notochord cells. This system has provided a powerful model to identify such targets, which play crucial role during zebrafish notochord morphogenesis.

The notochord abnormalities either in zebrafish embryos or any other animal was never been attributed to VPA and we also could not find any notochord abnormalities in zebrafish embryos when they were treated only with VPA even very high concentration (50 µM). The chemical modification in basic structure of VPA (by attaching chemical groups at various positions) drastically altered the biological activities of the resulting compounds. This affect has also been explained previously in many reports, where people have tried to synthesize VPA derivatives to minimize its toxicity (Nau & Loscher, 1986; Haj-Yehia & Bialer, 1989; Nau *et al.*, 1991; Bojic *et al.*, 1996; 1998; Radatz *et al.*, 1998; Lu *et al.*, 2004; Tasso *et al.*, 2004; Gravemann *et al.*, 2008).

Surprisingly V-ABE and V-ABH, which are structurally similar with V-AA, V-APA and V-ABA could

not produce abnormal notochord phenotypes; instead they induced gross abnormalities in developing embryos.

We have reported here the toxicity of five VPA structural analogous, which were newly synthesized. The comparative anti-angiogenic and HDAC inhibition profile between these compounds and VPA will be reported somewhere else.

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