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Research report

A larval zebrafish model of bipolar disorder as a screening platform for neuro-therapeutics

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HIGHLIGHTS

- Low-concentrations of GABA agonist PTZ produce multi-faceted changes in larval behaviour.
- Endophenotypes produced by PTZ mirror aspects of human psychological disorders.
- Seven known therapeutics that act on different targets were tested against the model.
- Each therapeutic showed a distinct effect on the behavioural pattern induced by PTZ.
- The larval zebrafish low-dose PTZ behavioural model will be useful for drug screening.

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ABSTRACT

Modelling neurological diseases has proven extraordinarily difficult due to the phenotypic complexity of each disorder. The zebrafish has become a useful model system with which to study abnormal neurological and behavioural activity and holds promise as a model of human disease. While most of the disease modelling using zebrafish has made use of adults, larvae hold tremendous promise for the high-throughput screening of potential therapeutics. The further development of larval disease models will strengthen their ability to contribute to the drug screening process. Here we have used zebrafish larvae to model the symptoms of bipolar disorder by treating larvae with sub-convulsive concentrations of the GABA antagonist pentylenetetrazol (PTZ). A number of therapeutics that act on different targets, in addition to those that have been used to treat bipolar disorder, were tested against this model to assess its predictive value. Carbamazepine, valproic acid, baclofen and honokiol, were found to oppose various aspects of the PTZ-induced changes in activity. Lidocaine and haloperidol exacerbated the PTZ-induced activity changes and sulpiride had no effect. By comparing the degree of phenotypic rescue with the mechanism of action of each therapeutic we have shown that the low-concentration PTZ model can produce a number of intermediate phenotypes that model symptoms of bipolar disorder, may be useful in modelling other disease states, and will help predict the efficacy of novel therapeutics.

1. Introduction

Numerous neurological and psychiatric disorders are associated with uncontrolled hyperactivity within different populations of neurons. The zebrafish has emerged as an attractive system for studying the behavioural consequences of aberrant neuronal activity induced by targeting various neuronal populations [7,9,12,24,35,50,53]. In addition to modelling seizures and epilepsy [3,6,7,23], zebrafish have also been used to study anxiety [38], and are predicted to be useful for studying other neuropsychiatric disorders, such as bipolar disorder and schizophrenia [10,40,47–49,55]. As such, the development of zebrafish behavioural models to study psychiatric disease has garnered much interest, both as a model to study disease etiology, and to aid in the development of new therapeutics [10,34].

The aim of modern therapeutic development has been to target specific populations of neurons, in order to treat the symptoms of a disease without producing negative side effects. This approach is hampered by both the behavioural complexity of most neuropsychiatric disorders, and the lack of appropriate models to test compound specificity.

Another approach has been to dissect the overt behavioural symptoms of a specific disorder into intermediate/endophenotypes; however, in humans this is exceedingly difficult. Animal models of intermediate disease phenotypes not only allow for a better understanding of disease states, but will also

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provide a platform with which to evaluate the specificity of potential therapeutics. While, mammalian models of endophenotypes have provided insights into disease symptomology, they lack the high-throughput ability required to screen the myriad of new chemical entities (NCEs) that are continuously being produced. The zebrafish has been shown to provide a link between the initial cell line screens and mammalian studies [34]. With the experimental advantages of the zebrafish, such as their low-cost, high-throughput and genetic tractability, the further development of predictive behavioural models in zebrafish will aide in the development of new therapeutics [10,13,19,24,29,30,47,56].

Chemically induced models of altered behaviour in zebrafish have shown the potential to generate models that replicated symptoms of psychiatric disorders. One example is the NMDA receptor antagonist MK-801, which has been suggested to act as a model of schizophrenia in adult zebrafish [47]. Recent work from our lab has described a behavioural model generated by targeting GABA receptors with low doses of the GABA receptor antagonist pentylenetetrazole (PTZ), that may act as a model of bipolar disorder [17].

Low, sub-convulsive, concentrations of PTZ produces a multifaceted behavioural profile in zebrafish larvae [17]. This profile consists of increases in fast-darting activity, elevations in anxiety/stress behaviours, a reversal of the normal response to cycles of light and dark, and the activation of the hypothalamic–pituitary–interrenal (HPI) axis. Anxiety, altered circadian activity and elevated activity in the mammalian equivalent of the HPI axis, the hypothalamic–pituitary–adrenal axis (HPA), have all been associated with bipolar disorder [25,26,43,45]. In addition, hyperlocomotion is one of the features that distinguishes bipolar disorder from schizophrenia [22,39,43]. It has also been reported that genetic variation in GABAA receptor expression contributes to the pathogenesis of psychiatric disorders, specifically, bipolar disorder [11,15,20]. In this way the low-concentration PTZ model appears to produce a number of intermediate phenotypes that model the symptoms of bipolar disorder and may be useful to measure the therapeutic potential of novel compounds.

In order to assess the usefulness of the low-concentration PTZ model as a screening platform for the identification of potential therapeutics, we have tested the model against a number of therapeutics that are used to treat the symptoms of bipolar disorder, along with novel compounds that target GABA receptors directly. Carbamazepine (CBZ), valproic acid (VPA) and lidocaine were compared with the antipsychotics sulpiride and haloperidol, along with the GABA agonists honokiol and baclofen. CBZ, VPA and baclofen were found to oppose some aspects of the PTZ-induced activity, while honokiol appeared to completely rescue the altered patterns of behaviour. Lidocaine and haloperidol exacerbated the PTZ-induced activity changes and sulpiride had no significant affect. By comparing the effect of each therapeutic on the activity pattern produced by low-concentrations of PTZ, we have demonstrated that this model can be useful for studying drug efficacy, predicting drug targets and aiding our understanding of disease etiology.

2. Materials and methods

2.1. Animals

Zebrafish (*Danio rerio*) were maintained according to standard animal care protocols [52] and in accordance with the Canadian Council on Animal Care guidelines. AB/Tubingen adults, embryos and larvae were maintained on a re-circulating Tecniplast aquatic system at 28 ± 1 °C and between pH 7.0–7.5 on a 14/10 h light/dark (L/D) cycle. Embryos were collected from multiple AB/Tubingen breeding pairs and pooled. Following 4–6 h in an incubator in E3 media (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2 \cdot 2H_2O$, 0.33 mM $MgSO_4$) unfertilised embryos were removed and the remainder placed in Aquatic Habitats mesh-bottom baby baskets on the recirculation system. This allowed the embryos to be maintained under consistent water conditions and L/D cycling matching the adults. Also, embryos raised on the system displayed more consistent activity patterns than those reared in a petri-dish.

2.2. Video tracking

Larvae were loaded into 96-well plates between 100 and 106 hpf (hours post fertilization), 1 fish/well with 270 μL HEPES buffered E3 (pH 7.2) and acclimated at 28 °C overnight. A 10× compound/drug solution was diluted into each well between 120 and 128 hpf and larvae were immediately placed into a Zebrabox (Viewpoint Life Sciences Inc., Montreal, Quebec, Canada) and their activity was monitored using the Viewpoint video tracking system and software. The plate temperature was maintained in the Zebrabox chamber at 28 °C by partial immersion in a recirculating water bath. All experiments consisted of 30 min of acclimation in the dark followed by 4–20 min cycles containing a light and dark phase (10 min each–110 min total). To capture the different types of activity a threshold was set at 20 mm/s in order to separate the fast/darting activity.

2.3. Data analysis

Data were analysed using GraphPad Prism software. The activity during the initial 5 min following a light to dark or dark to light transition was pooled for analysis. Replicate experiments were run on two separate days ($n = 12$/day) for both the therapeutic dilution series test and the PTZ dilution series test, with a carrier control group in each plate. One-way ANOVA was used to test for variability between treatment groups from each trial (95% confidence level). No significant differences were found allowing for the data from the replicates to be pooled for subsequent analysis ($n = 24$ or $n = 48$), and all treatment groups were compared to the pooled control data. Treatments were considered behaviourally toxic if they produced a reduction in activity over the course of the experiment, which was measured by comparing the activity from the 3 light/dark cycles (ANOVA, 95% confidence level). At concentrations in which there was no change in the activity level between the 3 light/dark cycles from the same experimental run, the data for the individual cycles were pooled for the light or dark phases and compared to controls. Significance was determined by a two-tailed Student's *t*-test assuming unequal variance.

3. Results

3.1. Characterization of known therapeutics

When 5-day-old zebrafish larvae are presented with short-repeated cycles of light and dark, they respond with an elevation in activity following a light to dark transition and a drop in activity following a dark–light transition [17,24]. At low concentrations of PTZ (between 2.5 and 5 mM), this activity pattern is reversed, a light–dark transition produces a drop in activity, while a dark–light transition produces an increase in activity. This change in activity is accompanied by an underlying increase in a fast-darting type of movement that can be quantified when a threshold is set (20 mm/s) to separate activity based on velocity. In order to further evaluate these behavioural changes and to test the usefulness of this model for identifying potential therapeutics, a number of known compounds with distinct mechanisms of action were tested for their ability to oppose these PTZ-induced changes in behaviour. Initially a concentration–response profile was generated for each therapeutic against a single concentration of PTZ (5 mM). A single dose of each therapeutic was then chosen and tested against the two doses of PTZ that produce the complex changes in behaviour (2.5 and 5 mM).

3.2. Testing anticonvulsants

Since, PTZ has previously been used to model seizure disorders in zebrafish [6], we initially tested the ability of three anticonvulsants on the low dose PTZ model, namely, carbamazepine (CBZ), valproic acid (VPA) and lidocaine.

CBZ has previously been shown to be toxic to zebrafish larvae at 300 μM [8], which led us to test the effect of CBZ at levels below this concentration. At 100 μM, CBZ showed a small, but significant, decrease in both the light and dark phase activity (−18.5% and −8%; Table 1). At 200 μM, CBZ produced a larger reduction in activity during both the light (−50%) and dark phases (−45.5%, Fig. 1A). Significance was determined by a two-tailed Student's *t*-test assuming

Table 1
Concentration response for known therapeutics versus a single concentration of PTZ (5 mM). Data presented as a fraction of control or PTZ-induced activity for three consecutive light/dark cycles. Red = significant increase in activity; green = significant decrease in activity (*t*-test, $p < 0.05$). ** denotes a decay in activity over the course of the experiment.

		Fraction of Control		Fraction of 5 mM PTZ		Fraction of 2.5 mM PTZ	
Therapeutic	**Concentration**	**Light**	**Dark**	**Light**	**Dark**	**Light**	**Dark**
CBZ	100 μM	0.82	0.92	1.12	1.34		
	200 μM	0.50	0.55	0.90	1.61	0.61	0.79
	250 μM**	0.38	0.27	0.69	1.30		
VPA	0.5 mM	0.80	0.87	0.72	0.83		
	1.5 mM	0.57	0.78	0.69	1.15	0.54	1.61
	3 mM**	0.30	0.67	0.51	0.86		
Lidocaine	50 μM	0.76	0.80	1.42	2.53		
	100 μM	0.57	0.71	1.38	2.43	1.35	2.31
	150 μM**	0.51	0.37	0.98	1.76		
Haloperidol	1 μM	1.10	1.05	1.26	1.51		
	2.5 μM	1.00	1.01	1.18	1.63		
	5 μM	1.11	1.00	1.14	1.54	1.72	2.83
Sulpiride	250 μM	0.93	0.98	1.21	1.18		
	500 μM	1.00	1.03	1.07	1.14		
	1 μM	0.92	0.96	1.08	1.13	1.11	1.05
Honokiol	1 μM	0.79	0.98	0.74	0.77		
	2.5 μM	0.61	0.87	0.48	0.96	0.79	2.55
	5 μM**	0.46	0.20	0.43	0.62		
Baclofen	100 μM	0.87	0.83	0.74	0.68		
	200 μM	0.82	0.67	0.59	0.51	0.63	0.88
	300 μM	0.70	0.56	0.42	0.43		

unequal variance and unless otherwise noted significant changes between treatment groups and controls will refer to differences producing $p < 0.05$. By 250 μM, CBZ produced a time-dependent decrease in normal activity over the course of the experiment, leading to what can be considered behavioural toxicity (data not shown).

When 100 μM CBZ was co-administered with PTZ (5 mM), there was no effect on the PTZ-induced change in larval activity during the light phase; however, there was an increase in activity associated with the dark phase (+34%). As the level of CBZ was elevated to 200 μM there was a small reduction in larval activity during the light phase (−11%) and an increase in activity during the dark phase (+60%, Fig. 1A, Table 1). The reduction in PTZ-induced light activity paired with the increase in PTZ-induced dark activity suggests that CBZ is opposing PTZ and may be moving the PTZ-induced larval activity pattern back to control levels. In order to further evaluate the ability of CBZ to oppose the PTZ-induced activity, we also tested this concentration of CBZ (200 μM) against a lower concentration of PTZ (2.5 mM), since a higher level of CBZ led to behavioural toxicity. This concentration of PTZ also produced a reversal in the normal response to light/dark transitions, albeit to a lesser degree. CBZ (200 μM) reduced the activity induced by 2.5 mM PTZ during both the light (39%) and dark (22%) phases (Fig. 1A, Table 1), suggesting that against this concentration of PTZ, CBZ is purely sedative.

In order to further assess the effect of CBZ on the PTZ-induced changes, a threshold was set (20 mm/s) in order to separate out the fast-darting activity that is a component of the complex phenotype produced by exposure to PTZ. CBZ had similar effects on the fast activity produced by PTZ as it did on the overall activity (Fig. 1B). The increase in fast activity, within the light and dark phases, produced by 2.5 mM PTZ was reduced by 200 μM CBZ (62% light decrease; 58.5% dark), while at 5 mM PTZ the level of fast activity was significantly reduced for the light phase (−16%) and elevated for the dark phase (+74%) by 200 μM CBZ.

While CBZ appeared to counter some of the changes induced by PTZ, VPA had a more dramatic effect. VPA is toxic to zebrafish larvae at levels above 3 mM [8], which was confirmed here by a reduction in activity over the course of the experiment (data not shown). At 500 μM, VPA significantly reduced normal activity and the larval activity induced by 5 mM PTZ during the light phase only (−28%; Table 1). At 1.5 mM, VPA reduced the normal activity during both the light and dark phases (−43% light, −22% dark, Fig. 1C). Interestingly, at 1.5 mM, VPA reduced the elevation in light phase activity (−31%) produced by 5 mM PTZ and produced a small (+15%), but not significant, increase in activity during the dark phase (Table 1, Fig. 1C). When the level of PTZ was reduced to 2.5 mM, 1.5 mM VPA was found to opposed the PTZ-induced change in activity by decreasing the level of activity during the light phase (−45.5%) and increasing the activity during the dark phase (+61%; Fig. 1C). This resulted in a return to the normal light/dark activity pattern produced by controls (dark activity > light activity) albeit at a lower overall activity level than controls.

VPA also opposed the increase in fast activity produced by PTZ, leading to a reduction in activity during the light phase (−78% at 2.5 mM; −30% at 5 mM, Fig. 1D). However, there was no significant effect on the elevation in fast activity during the dark phase induced by 2.5 or 5 mM PTZ (Fig. 1D).

The effect of lidocaine on larval activity was initially tested across a broad concentration range. At and above 150 μM, lidocaine leads to behavioural toxicity, represented by activity decay

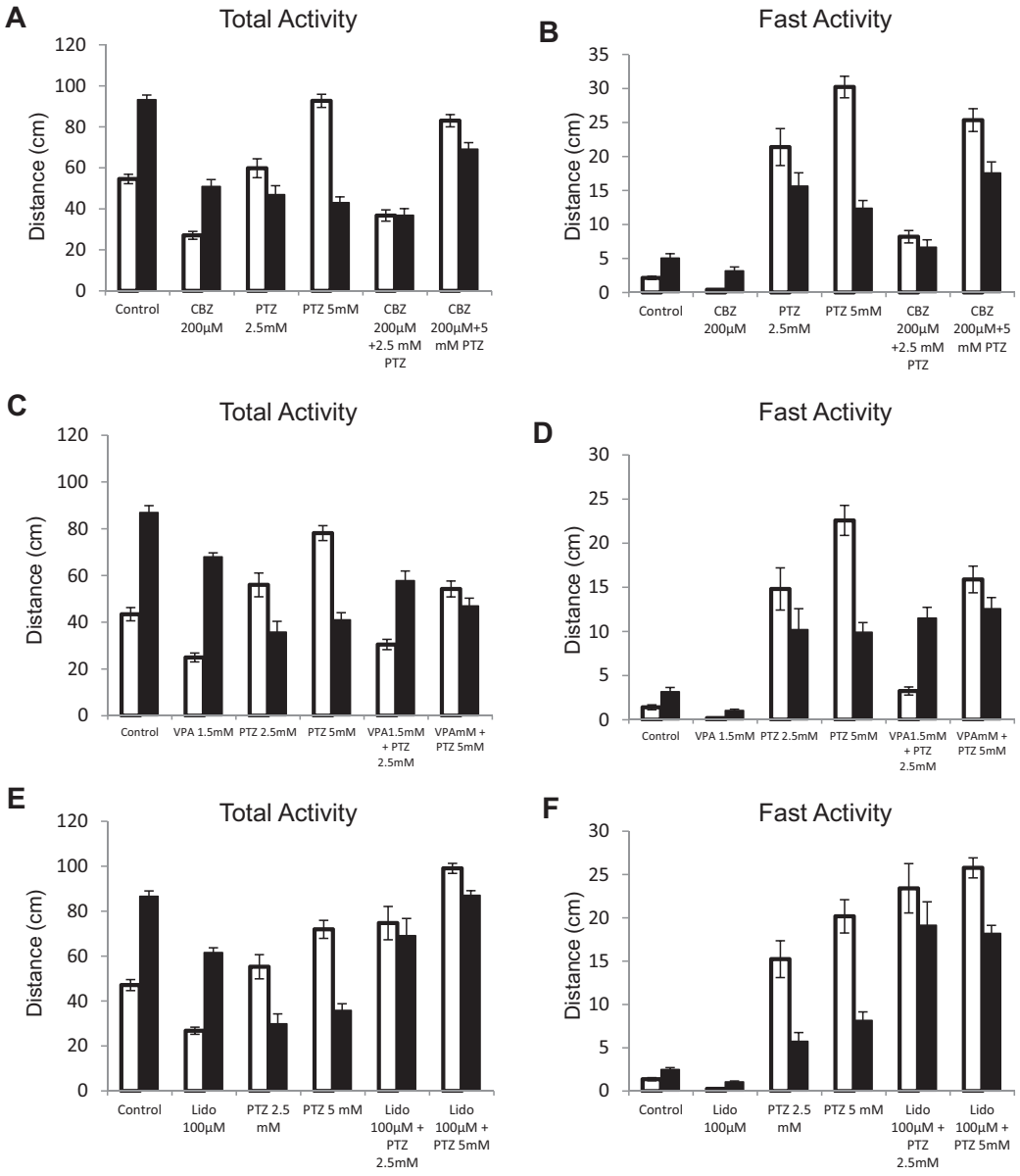

Fig. 1. Light (white bars) and dark (black bars) activity changes following treatment with PTZ and/or known therapeutics. (A) Carbamazepine (CBZ) treatment (200 μM) of activity changes produced by 2.5 and 5 mM PTZ. (B) A threshold was set to separate fast (over 20 mM/s) from total activity. (C and D) Valproic acid (VPA) treatment (1.5 mM) of PTZ-induced activity changes. (E and F) Lidocaine (Lido) treatment (100 μM) of PTZ-induced activity changes. Bars represent the average of three consecutive cycles of light–dark. Data presented as average distance travelled ± standard error; 5 min bins; $n = 24$/concentration.

over the course of the experiment (data not shown). There was a significant reduction in normal activity at 50 and100 μM (−24 and −44% reduction in light; −19 and −29% reduction in dark; Table 1) without behavioural toxicity. When paired with 5 mM PTZ, both 50 and 100 μM lidocaine led to an increase in activity during the light (+41 and +37%) and dark (+153 and +142%) phases over PTZ alone (Fig. 1E; Table 1). This increase in activity produced a profile in which there was no longer a change in activity levels between the light and dark phases, indicating that the larvae were no longer responsive to changes in light/dark. When 100 μM lidocaine was paired with a lower level of PTZ (2.5 mM), lidocaine still led to an increase in activity during both the light (+35%) and dark (+131%) phases, over PTZ alone (Fig. 1E). There was also an increase in fast activity levels when 100 μM lidocaine was co-administered with 2.5 (+54% light and +230% dark) or 5 mM (+46% light and +139% dark) PTZ (Fig. 1F).

3.3. Characterizing antipsychotics

We chose to test known anti-psychotics against the PTZ-induced activity changes. Sulpiride and haloperidol were chosen since they both target the dopaminergic system, which is often linked to psychological disorders, and have previously been shown to oppose MK-801 induced schizophrenia-like behaviours in adult zebrafish [47,48].

Haloperidol has previously been shown to lead to movement defects in zebrafish larvae resulting in decreases in activity at 9 μM [18]. Here we tested lower levels of haloperidol and found no significant difference over control activity levels at 1, 2.5 or 5 μM (Table 1). When co-administered with 5 mM PTZ, 1, 2.5 and 5 μM haloperidol increased the PTZ-induced activity by 26, 18 and 13% for the light phase and 50, 63 and 54% for the dark phase, respectively (Table 1, Fig. 2A). When 5 μM haloperidol was combined with

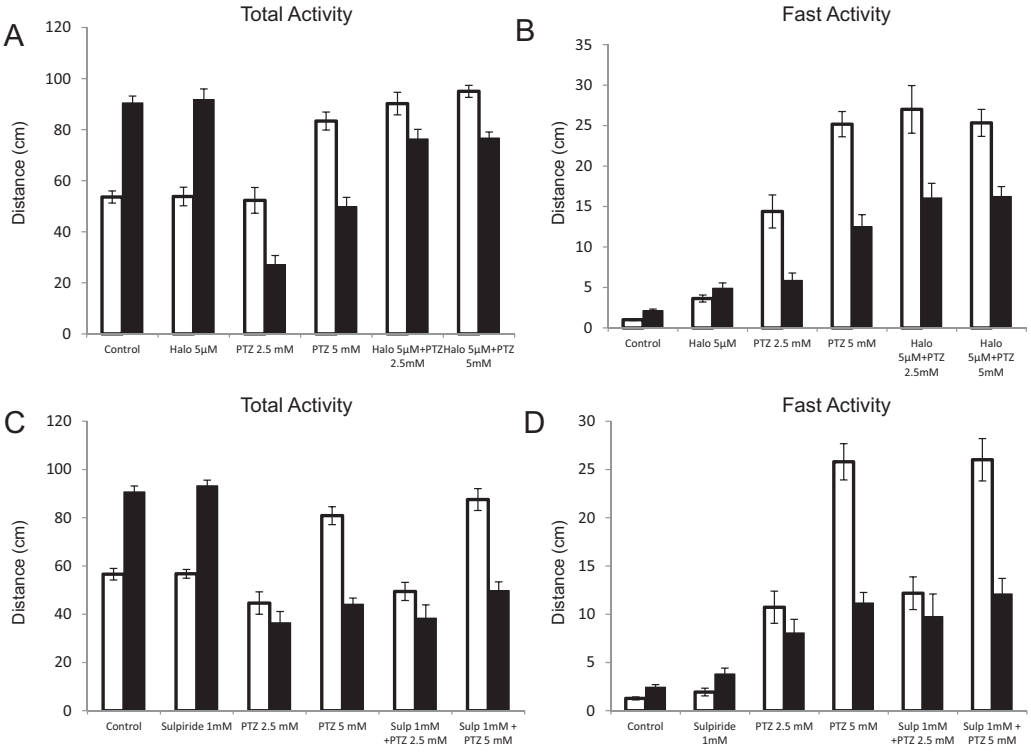

Fig. 2. Light (white bars) and dark (black bars) activity changes following treatment with PTZ and/or known therapeutics. (A and B) Haloperidol (Halo) treatment (5 μM) of activity changes produced by 2.5 and 5 mM PTZ. (C and D) Sulpiride (Sulp) treatment (1 mM) of PTZ-induced activity changes. Bars represent the average of three consecutive cycles of light–dark. Data presented as average distance travelled ± standard error; 5 min bins; $n = 24$/concentration.

5 mM PTZ there was a 20% reduction in activity between the first and third dark cycles that plateaued between the second and third cycles (data not shown). Since the activity plateaued and did not continue to decay it was not considered behaviourally toxic. When 5 μM haloperidol was paired with a lower level of PTZ (2.5 mM), there was also an increase in the level of activity, over PTZ alone (+72% light; +182% dark, Fig. 2A). The fast activity produced by 5 μM haloperidol combined with 2.5 mM PTZ was also higher than that produced by PTZ alone (+88% light; +173% dark, Fig. 2B). While the fast activity produced by 5 μM haloperidol combined with 5 mM PTZ was not significantly different than 5 mM PTZ alone, when the 3 light/dark cycles were averaged (Fig. 2B). However, there was an underlying decrease in activity between the first and third cycles for the fast activity produced by 5 μM haloperidol/5 mM PTZ. Unlike the overall activity, the fast activity decayed below control levels by the third cycle (data not shown). This suggests that the initial drop in activity that led to the plateau in overall activity is due to the large decay in fast activity.

Previous studies have shown that at 250 μM sulpiride opposed MK-801 induced changes in activity in adult zebrafish [46–48]. In the current study, 250 μM sulpiride had no significant effect on the normal activity, but did lead to an increase in the PTZ-induced activity (5 mM) during the light phase (+20%; Table 1). Higher levels of sulpiride (up to 1 mM) did not have a significant effect on the activity induced by 2.5 or 5 mM PTZ (Fig. 2C and D; Table 1).

3.4. GABA targeting compounds

Since PTZ is a known GABA antagonist we tested compounds that act on GABA receptors for their ability to oppose PTZ-induced activity. Honokiol produced a significant decrease in larval activity during the light phase at 1 μM (−21%), and decreases in activity during both the light and dark phases at 2.5 and 5 μM (−49 and −54% light, −13 and −80% dark, Table 1). Treatment with 5 μM honokiol resulted in an activity decay over the course of the experiment, and was thus considered behaviourally toxic (data not shown).

At 1 μM, honokiol reduced the activity produced by 5 mM PTZ during the light phase (−24%) and also reduced the level of dark phase activity (−23%), although the reduction in dark phase activity was not statistically significant. While 1 μM honokiol produced a general reduction in the PTZ-induced activity pattern, 2.5 μM honokiol partially reversed the light/dark switch produced by 5 mM PTZ, by decreasing the activity during the light phase (−52%). This brought the light phase activity level below that of the dark (Table 1, Fig. 3A). Since 5 μM honokiol was behaviourally toxic, and 2.5 μM honokiol partially rescued the light/dark response, 2.5 μM was subsequently tested against a lower level of PTZ. At 2.5 μM, honokiol reduced the larval activity produced by 2.5 mM PTZ during the light phase (−21%) and increased the activity during the dark phase (+154%) returning the activity back to a level and pattern that was not significantly different than controls (Table 1, Fig. 3A).

Not only did honokiol oppose the PTZ-induced reversal of the light/dark cycling pattern, it also eliminated the increase in fast activity produced by PTZ, at 2.5 and 5 mM, to levels that were not significantly different from carrier controls (−93 and −88% light; −36.5 and −73% dark). These results suggest that honokiol can rescue both the light/dark activity pattern changes and oppose the elevated fast/darting activity produced by PTZ.

In order to assess if the GABA receptor effect was subtype specific we also tested the $GABA_B$ agonist baclofen. Baclofen treatment produced a general decrease in larval activity on its own, reducing the activity levels during the dark phase at 100 μM (−17.5%) and during the light and dark phases at 200 and 300 μM (−18 and −30% light; −33 and −45% dark; Table 1). Baclofen did not affect the changes in the pattern of light/dark cycling, but similar to the effect on normal activity led to an overall decrease in the activity induced

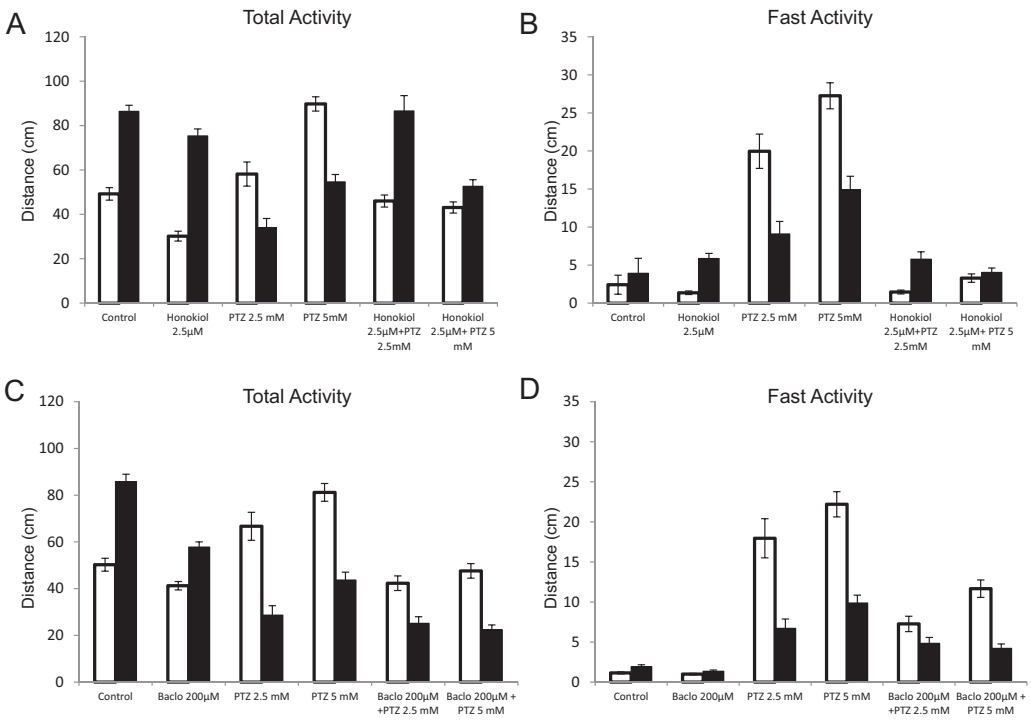

Fig. 3. Light (white bars) and dark (black bars) activity changes following treatment with PTZ and/or known therapeutics. (A and B) Honokiol (Honok) treatment (2.5 μM) of activity changes produced by 2.5 and 5 mM PTZ. (C and D) Baclofen (Baclo) treatment (200 μM) of PTZ-induced activity changes. Bars represent the average of three consecutive cycles of light–dark. Data presented as average distance travelled ± standard error; 5 min bins; $n = 24$/concentration.

by 5 mM PTZ (Table 1, Fig. 3C). At 2.5 mM PTZ, 200 μM baclofen reduced the elevated activity during the light phase (−41.5%) but did not affect the activity during the dark phase. Baclofen also reduced the fast activity produced by 2.5 and 5 mM PTZ in a similar manner to that of the total activity (−24.5 and 64.5% light; −38 and 54.5% dark). The fast activity levels remained higher than the controls (Fig. 3D).

4. Discussion

In recent years the development of new therapeutics to treat complex neuropathies has stalled. While countless libraries of NCEs can be created and their specificity can initially be measured in vitro, downstream in vivo studies can reveal either a lack of activity or off-target side effects. Since the mammalian in vivo testing methods currently in use are costly, there is a requirement for new, high-throughput, in vivo methods to help bridge the gap between the initial in vitro analysis and the mammalian testing. Importantly, it will be useful for these models to not only screen for toxic and off-target effects, but also to provide information that would allow predictions to be made regarding the therapeutic potential of new compounds. In recent years, zebrafish drug-screening techniques have been developed that may provide this link [34].

In order to complement the larval zebrafish models that test the effects of compounds on their own, we have begun to evaluate the predictive value of a previously developed model of disease symptomology. Our previous work has described a low-concentration PTZ model that produces a multifaceted behavioural profile [17]. Within a sub-convulsive concentration range, PTZ increases fast-darting activity, elevates anxiety/stress behaviours (i.e. thigmotaxis), and reverses the normal response to cycles of light and dark. Anxiety, altered circadian activity and hyperlocomotion, have all been associated with bipolar disorder [22,25,26,39,43,45]. In addition, PTZ acts as a GABA receptor agonist and links are beginning to be made between GABA receptor expression and psychiatric disorders, such as bipolar disorder [11,15,20]. In this way the low-concentration PTZ model appears to produce a number of intermediate phenotypes that may be useful when assessing the efficacy of potential therapeutic compounds.

We have expanded upon our previous work by attempting to oppose the altered activity patterns produced by low-concentrations of PTZ with different classes of therapeutics, in order to assess its predictive value. It appears that not only can this model predict potentially efficacious therapeutics, but it can also reveal unexpected negative side effects that may not be found when screening the compound on its own.

The testing of therapeutics began with two compounds that have been used to treat bipolar disorder in humans, CBZ and VPA [21,28,42]. Both have also been used extensively to treat anxiety [4] and the mania associated with psychological disorders in humans [21,44] and rodents [5]. In this study both compounds were found to oppose components of the PTZ-induced changes in behaviour. Importantly, while both compounds reduced the level of hyperactivity induced by PTZ, VPA also led to a partial rescue of the reversal in the light/dark response that was produced by PTZ. This returned the cycling pattern back to one in which the larval activity during the dark phase is higher than during the light phase.

CBZ primarily acts as an inhibitor of voltage-gated sodium channels (VGSCs), and likely opposes PTZ-induced activity through a general, non-specific decrease in neural activity, as found in a rodent model of mania [5]. VPA acts on a number of targets including GABA, glutamine, VG sodium and calcium channels [25]. This would allow VPA to oppose the PTZ-induced activity through both a general decrease in activity and by decreasing the antagonistic effect of PTZ on GABA receptor activity directly. Overall, VPA appeared to provide a stronger opposition to the PTZ-induced changes in activity than CBZ. This would seem to reflect their previously defined therapeutic potential, as VPA or lithium are recommended as first line treatments of bipolar disorder, while CBZ is only recommended as a second line treatment [42].

One of the main differences between VPA and CBZ activity is the effect of VPA on the GABAergic system. It has been reported that genetic variation in GABAA receptor expression contributes to the pathogenesis of bipolar disorder [11,15,20]. The link between the behavioral changes produced by PTZ in this study and its proposed effect on the GABAA receptor would seem to be confirmed by the phenotypic rescue with the GABAA receptor agonist honokiol [51]. In fact, it appears that honokiol can completely rescue the altered larval activity induced by PTZ. Honokiol is a naturally-derived bioactive obtained from the magnolia tree, and has anti-convulsant [14,36,37] and anoxilytic properties in rodent models [31,37]. Here we have found that in addition to reducing the PTZ-induced fast activity, honokiol also returns the light/dark cycling activity to a pattern similar to controls. The light/dark pattern produced by 2.5 mM PTZ is not only reversed by honokiol, but the activity levels are also returned to those of controls, suggesting a complete rescue of the PTZ-induced phenotype. This effect was not found for the GABAB agonist baclofen, which acted in a purely sedative fashion. Therapeutics that target $GABA_A$ receptors, such as benzodiazepines and barbiturates, have relatively large side effect profiles, when use to treat psychiatric disorders [16]. This makes compounds, such as honokiol, that target GABA receptors, and have a lower side effect profile, of interest for treating psychiatric disorders [2,33,51].

In addition to assessing the therapeutic potential of novel compounds, the PTZ model may also reveal side effects that would not otherwise be noted. Lidocaine, which has been used to treat refractory cases of epilepsy, did not oppose the PTZ-induced activity, but rather appeared to exacerbate it. It has been suggested that lidocaine can actually induce seizures and may do so by acting on inhibitory circuits [1,32,54]. This may indicate that lidocaine amplifies the decreased inhibition produced by PTZ's antagonistic effect on GABA receptor activity, leading to a synergistic effect. Since, on its own, lidocaine reduced the normal activity of larvae, it appears that the PTZ model may be able to predict side effects that would not have otherwise been revealed.

The antipsychotics sulpiride and haloperidol were selected due to their ability to counteract the elevation in activity of adult zebrafish produced by the NMDA antagonist MK-801, a proposed model of schizophrenia [47]. Here we have shown that neither compound can counteract the PTZ-induced changes in activity. On the contrary, similar to lidocaine, haloperidol intensified the larval response to PTZ. Unlike lidocaine, however, haloperidol on its own increased larval activity. Interestingly haloperidol has a side effect profile that includes movement disorders such as akathisia, parkinsonism and dystonia [27], which may be partially replicated here. The other anti-psychotic compound tested, sulpiride, a dopaminergic receptor antagonist, had virtually no effect on PTZ-induced activity changes, suggesting that they are not directly linked to the activity of dopaminergic neurons.

Another key component for a diagnosis of bipolar disorder is an alteration in circadian activity [26,41]. Preliminary evidence from our lab suggests that at the concentrations of PTZ that produce a reversal in the normal response to short light/dark cycles there is also a reversal in the normal activity levels found during prolonged periods of light and dark [17]. While more work is required to confirm that this reversal truly represents a change in circadian activity, it appears that at least some aspects of the normal circadian response are altered.

Care must be taken when correlating the activity of larval zebrafish to complex psychological disorders; however, at present no strong animal models exist (see [22]). Modelling intermediate phenotypes associated with these complex disorders may provide an alternative mechanism for the identification of efficacious therapies for these disorders. In particular, modulation of the intermediate phenotypes produced by PTZ may allow for predictions to be made regarding the therapeutic potential of novel compounds for the treatment of GABA-mediated behaviours. This is highlighted by the finding that honokiol, a compound not currently used for the treatment of complex psychiatric disorders, can oppose the PTZ-induced activity patterns.

Models such as the one described here can potentially go beyond the initial assessment of therapeutics (i.e. toxicity) and begin to test their efficacy against models of intermediate disease phenotypes. Other zebrafish models have been proposed to model intermediate phenotypes that may be associated with complex psychological disorders, such as, anxiety [38] and schizophrenia [47,48]. This will provide multiple platforms with which to test potential therapeutics and may help to shed light on the pathology of these complex disease states.

Conflicts of interest

The authors declare no conflicts of interest.

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References

[1] Abed WT. Alterations of lidocaine and pentylenetetrazol-induced convulsions by manipulation of brain monoamines. Pharmacology & Toxicology 1994;75:162–5.
[2] Ai J, Wang X, Nielsen M. Honokiol and magnolol selectively interact with GABAA receptor subtypes in vitro. Pharmacology 2001;63:34–41.
[3] Alfaro JM, Ripoll-Gomez J, Burgos JS. Kainate administered to adult zebrafish causes seizures similar to those in rodent models. European Journal of Neuroscience 2011;33:1252–5.
[4] Aliyev NA, Aliyev ZN. Valproate (depakine-chrono) in the acute treatment of outpatients with generalized anxiety disorder without psychiatric comorbidity: randomized double-blind placebo-controlled study. European Psychiatry 2008;23:109–14.
[5] Arban R, Maraia G, Brackenborough K, Winyard L, Wilson A, Gerrard P, et al. Evaluation of the effects of lamotrigine valproate and carbamazepine in a rodent model of mania. Behavioural Brain Research 2005;158:123–32.
[6] Baraban SC, Dinday MT, Castro PA, Chege S, Guyenet S, Taylor MR. A large-scale mutagenesis screen to identify seizure-resistant zebrafish. Epilepsia 2007;48:1151–7.
[7] Baraban SC, Taylor MR, Castro PA, Baier H. Pentylenetetrazole induced changes in zebrafish behavior neural activity and c-fos expression. Neuroscience 2005;131:759–68.
[8] Berghmans S, Hunt J, Roach A, Goldsmith P. Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants. Epilepsy Research 2007;75:18–28.
[9] Blaser R, Gerlai R. Behavioral phenotyping in zebrafish: comparison of three behavioral quantification methods. Behavior Research Methods 2006;38:456–69.
[10] Brennan CH. Zebrafish behavioural assays of translational relevance for the study of psychiatric disease. Reviews in the Neurosciences 2011;22:37–48.
[11] Breuer R, Hamshere ML, Strohmaier J, Mattheisen M, Degenhardt F, Meier S, et al. Independent evidence for the selective influence of GABA(A) receptors on one component of the bipolar disorder phenotype. Molecular Psychiatry 2011;16:587–9.
[12] Burgess HA, Granato M. Modulation of locomotor activity in larval zebrafish during light adaptation. Journal of Experimental Biology 2007;210:2526–39.
[13] Chakraborty C, Hsu CH, Wen ZH, Lin CS, Agoramoorthy G. Zebrafish: a complete animal model for in vivo drug discovery and development. Current Drug Metabolism 2009;10:116–24.
[14] Chang-Mu C, Jen-Kun L, Shing-Hwa L, Shoei-Yn LS. Characterization of neurotoxic effects of NMDA and the novel neuroprotection by phytopolyphenols in mice. Behavioral Neuroscience 2010;124:541–53.
[15] Craddock N, Jones L, Jones IR, Kirov G, Green EK, Grozeva D, et al. Strong genetic evidence for a selective influence of GABAA receptors on a component of the bipolar disorder phenotype. Molecular Psychiatry 2010;15:146–53.
[16] Dell'osso B, Lader M. Do benzodiazepines still deserve a major role in the treatment of psychiatric disorders? A critical reappraisal. European Psychiatry 2012 [Epub ahead of print].
[17] Ellis LD, Seibert J, Soanes KH. Distinct models of induced hyperactivity in zebrafish larvae. Brain Research 2012;1449:46–59.

[18] Giacomini NJ, Rose B, Kobayashi K, Guo S. Antipsychotics produce locomotor impairment in larval zebrafish. Neurotoxicology and Teratology 2006;28:245–50.
[19] Goldsmith P. Zebrafish as a pharmacological tool: the how, why and when. Current Opinion in Pharmacology 2004;4:504–12.
[20] Green EK, Grozeva D, Moskvina V, Hamshere ML, Jones IR, Jones L, et al. Variation at the GABAA receptor gene, Rho 1 (GABRR1) associated with susceptibility to bipolar schizoaffective disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 2010;153B:1347–9.
[21] Grunze HC. Anticonvulsants in bipolar disorder. Journal of Mental Health 2010;19:127–41.
[22] Henry BL, Minassian A, Young JW, Paulus MP, Geyer MA, Perry W. Cross-species assessments of motor and exploratory behavior related to bipolar disorder. Neuroscience and Biobehavioral Reviews 2010;34: 1296–306.
[23] Hortopan GA, Dinday MT, Baraban SC. Zebrafish as a model for studying genetic aspects of epilepsy. Disease Models & Mechanisms 2010;3:144–8.
[24] Irons TD, MacPhail RC, Hunter DL, Padilla S. Acute neuroactive drug exposures alter locomotor activity in larval zebrafish. Neurotoxicology and Teratology 2010;32:84–90.
[25] Johannessen Landmark C. Antiepileptic drugs in non-epilepsy disorders: relations between mechanisms of action and clinical efficacy. CNS Drugs 2008;22:27–47.
[26] Jones SH. Circadian rhythms multilevel models of emotion and bipolar disorder—an initial step towards integration? Clinical Psychology Review 2001;21:1193–209.
[27] Joy CB, Adams CE, Lawrie SM. Haloperidol versus placebo for schizophrenia. Cochrane Database System Review 2006:CD003082.
[28] Kaufman KR. Antiepileptic drugs in the treatment of psychiatric disorders. Epilepsy Behavior 2011;21:1–11.
[29] Kokel D, Bryan J, Laggner C, White R, Cheung CY, Mateus R, et al. Rapid behavior-based identification of neuroactive small molecules in the zebrafish. Nature Chemical Biology 2010;6:231–7.
[30] Kokel D, Peterson RT. Chemobehavioural phenomics and behaviour-based psychiatric drug discovery in the zebrafish. Briefing in Functional Genomics and Proteomics 2008;7:483–90.
[31] Kuribara H, Stavinoha WB, Maruyama Y. Behavioural pharmacological characteristics of honokiol an anxiolytic agent present in extracts of Magnolia bark, evaluated by an elevated plus-maze test in mice. Journal of Pharmacy and Pharmacology 1998;50:819–26.
[32] Kurt M, Bilge SS, Kukula O, Kesim Y, Celik S. The role of nitrergic system in lidocaine-induced convulsion in the mouse. Japanese Journal of Pharmacology 2001;85:92–4.
[33] Lee YJ, Lee YM, Lee CK, Jung JK, Han SB, Hong JT. Therapeutic applications of compounds in the Magnolia family. Pharmacology & Therapeutics 2011;130:157–76.
[34] Levin ED. Zebrafish assessment of cognitive improvement and anxiolysis: filling the gap between in vitro and rodent models for drug development. Reviews in the Neurosciences 2011;22:75–84.
[35] Li X, Ricci R, Large CH, Anderson B, Nahas Z, George MS. Lamotrigine and valproic acid have different effects on motorcortical neuronal excitability. Journal of Neural Transmission 2009;116:423–9.
[36] Lin YR, Chen HH, Ko CH, Chan MH. Differential inhibitory effects of honokiol and magnolol on excitatory amino acid-evoked cation signals and NMDA-induced seizures. Neuropharmacology 2005;49:542–50.
[37] Martinez AL, Dominguez F, Orozco S, Chavez M, Salgado H, Gonzalez M, et al. Neuropharmacological effects of an ethanol extract of the *Magnolia dealbata* Zucc. leaves in mice. Journal of Ethnopharmacology 2006;106: 250–5.
[38] Maximino C, de Brito TM, da Silva Batista AW, Herculano AM, Morato S, Gouveia Jr A. Measuring anxiety in zebrafish: a critical review. Behavioural Brain Research 2010;214:157–71.
[39] Minassian A, Henry BL, Young JW, Masten V, Geyer MA, Perry W. Repeated assessment of exploration and novelty seeking in the human behavioral pattern monitor in bipolar disorder patients and healthy individuals. PLoS One 2011;6:e24185.
[40] Morris JA. Zebrafish: a model system to examine the neurodevelopmental basis of schizophrenia. Progress in Brain Research 2009;179:97–106.
[41] Murray G, Harvey A. Circadian rhythms and sleep in bipolar disorder. Bipolar Disorders 2010;12:459–72.
[42] Nivoli AM, Murru A, Goikolea JM, Crespo JM, Montes JM, Gonzalez-Pinto A, et al. New treatment guidelines for acute bipolar mania: a critical review. Journal of Affective Disorders 2011;129(1–3):14–26.
[43] Perry W, Minassian A, Henry B, Kincaid M, Young JW, Geyer MA. Quantifying over-activity in bipolar and schizophrenia patients in a human open field paradigm. Psychiatry Research 2010;178:84–91.
[44] Rosa AR, Fountoulakis K, Siamouli M, Gonda X, Vieta E. Is anticonvulsant treatment of mania a class effect? Data from randomized clinical trials. CNS Neuroscience & Therapeutics 2011;17:167–77.
[45] Salvadore G, Quiroz JA, Machado-Vieira R, Henter ID, Manji HK, Zarate Jr CA. The neurobiology of the switch process in bipolar disorder: a review. Journal of Clinical Psychiatry 2010;71:1488–501.
[46] Savio LE, Vuaden FC, Piato AL, Bonan CD, Wyse AT. Behavioral changes induced by long-term proline exposure are reversed by antipsychotics in zebrafish. Progress in Neuro-psychopharmacology Biological Psychiatry 2012;36:258–63.
[47] Seibt KJ, Oliveira RD, Zimmermann FF, Capiotti KM, Bogo MR, Ghisleni G, et al. Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (*Danio rerio*). Behavioural Brain Research 2010;214:417–22.
[48] Seibt KJ, Piato AL, da Luz Oliveira R, Capiotti KM, Vianna MR, Bonan CD. Antipsychotic drugs reverse MK-801-induced cognitive and social interaction deficits in zebrafish (*Danio rerio*). Behavioural Brain Research 2011;224: 135–9.
[49] Souza BR, Tropepe V. The role of dopaminergic signalling during larval zebrafish brain development: a tool for investigating the developmental basis of neuropsychiatric disorders. Reviews in the Neurosciences 2011;22: 107–19.
[50] Steenbergen PJ, Richardson MK, Champagne DL. The use of the zebrafish model in stress research. Progress in Neuro-psychopharmacology Biological Psychiatry 2010;35:1432–51.
[51] Taferner B, Schuehly W, Huefner A, Baburin I, Wiesner K, Ecker GF, et al. Modulation of GABAA-receptors by honokiol and derivatives: subtype selectivity and structure-activity relationship. Journal of Medicinal Chemistry 2011;54:5349–61.
[52] Westerfield M. The zebrafish book: a guide for laboratory us of zebrafish (*Danio rerio*). Eugene, OR: University of Oregon Press; 1995.
[53] Winter MJ, Redfern WS, Hayfield AJ, Owen SF, Valentin JP, Hutchinson TH. Validation of a larval zebrafish locomotor assay for assessing the seizure liability of early-stage development drugs. Journal of Pharmacological and Toxicological Methods 2008;57:176–87.
[54] Yoshimura Y, Dohi T, Tanaka S, Takada K, Tsujimoto A. Changes in convulsion susceptibility of lidocaine by alteration of brain catecholaminergic functions. Japanese Journal of Pharmacology 1991;56:85–91.
[55] Zakhary SM, Ayubcha D, Ansari F, Kamran K, Karim M, Leheste JR, et al. A behavioral and molecular analysis of ketamine in zebrafish. Synapse 2011;65:160–7.
[56] Zon LI, Peterson R. The new age of chemical screening in zebrafish. Zebrafish 2010;7:1.