Chapter 4

SYNTHESIS AND CHARACTERIZATION OF N’-(2-(SUBLITUTED BENZAMIDO)THIAZOL-4-YL)-2-PROPYLENEDIAMINEHYDRAZIDE
4.1 INTRODUCTION

Valproic acid is simple branched chain carboxylic acid used in epilepsy. Valproic acid increases GABA (γ-amino butyric acid) synthesis and release and potentiates by this mechanism GABA ergic transmission in specific brain regions. Valproic acid also reduces the release of excitatory amino acid β-hydroxy butyric acid and attenuates neuronal excitation mediated by activation of N-methyl-D-aspartames glutamate receptors. Valproic acid is a broad-spectrum antiepileptic drug effective against all seizure types.

Valproic acid (chemical name 2-propylvaleric acid) was first synthesized in 1882 by Burton as an analogue of valeric acid, found naturally in valerian. A clear liquid fatty acid at room temperature, for many decades its only use was in laboratories as a "metabolically inert" solvent for organic compounds. In 1962, the French researcher Pierre Eymard serendipitously discovered the anticonvulsant properties of valproic acid while using it as a vehicle for a number of other compounds that were being screened for anti-seizure activity. He found that it prevented pentylenetetrazol-induced convulsions in rodents. Since then it has also been used for migraine and bipolar disorder.

4.2 VALPROATE: PAST, PRESENT, AND FUTURE

Preclinical studies have been carried out during the past four decades to investigate the different mechanisms of action of valproate (VPA). The mechanisms of VPA which seem to be of clinical importance include increased GABA ergic activity, reduction in excitatory neurotransmission, and modification of monoamines. These mechanisms are discussed in relation to the various clinical uses of the drug. VPA is widely used as an antiepileptic drug with a broad spectrum of activity. In patients, VPA possesses efficacy in the treatment of various epileptic seizures such as absence, myoclonic, and generalized tonic-clonic seizures. It is also effective in the treatment of partial seizures with or without secondary generalization and acutely in status epileptics. The pharmacokinetic aspects of VPA and the frequent drug interactions between VPA and other drugs are discussed. The available methods for the determination of VPA in body fluids are briefly evaluated. At present, investigations
and clinical trials are carried out and evaluated to explore the new indications for VPA in other conditions such as in psychiatric disorders, migraine and neuropathic pain. Furthermore, the toxicity of VPA, both regarding commonly occurring side effects and potential idiosyncratic reactions are described. Derivatives of VPA with improved efficacy and tolerability are in development.

4.3 CHEMICAL STRUCTURE OF VALPROIC ACID AND ITS DERIVATIVES

Molecular formula of valproic acid is $\text{C}_8\text{H}_{16}\text{O}_2$ and molecular weight is 144.2. The derivatives of valproic acid are Sodium valproate molecular formula is $\text{C}_8\text{H}_{15}\text{NaO}_2$ and molecular weight is 166.2, Semi sodium valproate molecular formula is $\text{C}_{16}\text{H}_{31}\text{NaO}_4$ and molecular weight is 310.4, Valproate pivoxil molecular formula is $\text{C}_{14}\text{H}_{26}\text{O}_4$ and molecular weight is 258.4, Valpromide molecular formula is $\text{C}_8\text{H}_{17}\text{NO}$ and molecular weight is 143.2.

**Chemical names of Valproic acid:**
2-Propylpentanoic acid, 2-Propylvaleric acid, Di-n-dipropylacetic acid

**Chemical names of Sodium valproate:**
Sodium 2-propylvalerate, Sodium 2-propylpentanoate

**Chemical names of Semi sodium valproate:**
2-Propylvaleric acid-sodium 2-propylvalerate, Sodium hydrogen bis(2-propylvalerate)

**Chemical names of Valproate pivoxil:**
Hydroxymethyl 2-propylvalerate pivalate

**Chemical names of Valpromide:**
Dipropylacetamide, 2-Propylvaleramide
4.4 PHYSICAL PROPERTIES OF VALPROIC ACID

Valproic acid is colorless to pale yellow viscous liquid. It is slightly soluble in water (1.2 mg/mL); fully soluble in acetone, chloroform, ether and methyl alcohol. Valproic acid is stored in airtight containers and is sensitive to light. Valproic acid capsules should be stored at 15 to 30 °C and freezing should be avoided.

4.5 USES OF VALPROIC ACID

Valproic acid is used solely or in combination with other anticonvulsants in the treatment of simple (petit mal) and complex absence seizures. Valproate may be effective against myoclonic and atonics seizures in young children.

4.6 SYNTHESIS OF VALPROIC ACID

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4.7 MECHANISM ACTION OF VALPROIC ACID

Valproic acid (VPA) is indicated for the treatment of epilepsy and bipolar disorder and in the prevention of migraine headaches. VPA has also become more widely prescribed due to several off-label indications such as in the treatment of neuropathic pain and cancer.\textsuperscript{1,2} Despite VPA being well tolerated and having a low incidence of serious side effects, one concern with VPA therapy is weight gain. A prospective study identified that 37% of female patients with epilepsy developed obesity, as defined as a body mass index (BMI) greater than 25, after 1 yr of treatment with VPA.\textsuperscript{3} Numerous retrospective and cross-sectional analyses also report that treatment with VPA is associated with a significant increase in weight ranging from 5 to 49 kg.\textsuperscript{4,5,6,7} Studies examining VPA-induced weight gain have been conducted predominantly in adult women because VPA can induce a number of reproductive endocrine abnormalities that include hyperandrogenism, menstrual disturbances, weight gain, and/or polycystic ovaries.\textsuperscript{8,9,10} Fifty-two percent of males treated with VPA, however, also have BMI scores within the obesity category,\textsuperscript{11} and youth and adolescents treated with VPA are reported to have BMI scores over expected age norms.\textsuperscript{12,13,14} Similarly, a prospective double-blind comparison of the incidence and magnitude of weight gain in patients receiving VPA. Lamotrigine monotherapy demonstrated that weight gain was greater for those patients treated with VPA and was significant within 10 wk of treatment onset.\textsuperscript{15,16} Weight gain associated with VPA treatment is of great concern due to its physical and psychological consequences.\textsuperscript{17} Notably, obesity leads to increase risk for numerous other diseases, such as diabetes mellitus, coronary heart disease,\textsuperscript{18} and increased noncompliance with pharmacotherapy in psychiatric patients.\textsuperscript{19}

The mechanism underlying VPA-induced weight gain has not been elucidated. Age, gender, medical condition, dose and serum concentrations of VPA and family history of body weight problems are not significantly correlated with the gain in weight associated with VPA treatment.\textsuperscript{20,21} In attempts to generate animal models of VPA-induced weight gain, VPA has been shown to induce a significant increase in body weight in female rhesus monkeys;\textsuperscript{22} however, we and others have demonstrated that VPA does not cause weight gain in rodents.\textsuperscript{23,24,25} The etiology of VPA-induced weight gain is most likely multifactorial because weight is the output of energy homeostasis controlled by many organs that produce and secrete a variety of appetite-
regulating peptides and cytokines that act within the hypothalamus. VPA treatment in humans increases the serum level of two hormones, leptin and insulin, which are produced by the adipose tissue and pancreatic β-cells, respectively. After VPA treatment for 1 yr, 37% of female patients with epilepsy who developed obesity had a 1.8-fold increase in fasting serum insulin and 3.4-fold increase in serum leptin levels. Similarly, in women receiving VPA for treatment of bipolar disorder, insulin and leptin levels were significantly elevated when compared with women receiving lithium. High levels of serum leptin are commonly associated with obesity and could represent a state of leptin resistance. The increase in serum leptin associated with weight gain after VPA treatment may be a consequence of the increase in adipose tissue; however, it is also possible that VPA may have a direct effect on leptin secretion from adiposities or may alter leptin signaling and decrease negative feedback. VPA has been shown to have direct effects on hormone secretion from other endocrine cells. For example, an ex vivo study using human pancreatic islet cells has shown that VPA can directly increase insulin release. Moreover, VPA can also potentiate androgen production from ovarian theca cells. We previously demonstrated that VPA inhibited mouse 3T3-L1 and human preadipocyte differentiation. Treatment with VPA during adipogenesis reduced the protein levels for several key adipocyte-specific transcription factors, including CCAAT/enhancer binding protein (C/EBP)-α, peroxisome proliferators-activated receptor (PPAR)-γ, and steroid regulatory element binding protein (SREBP) 1a. The present work demonstrates that treatment with VPA in mature adiposities significantly reduces leptin mRNA levels and secretion of the leptin protein in a dose and time-dependent manner. These findings were paradoxical because treatment of patients with VPA is associated with increased serum leptin levels. The reduction in leptin secretion from adiposities was not accompanied by alterations in glucose uptake or altered intracellular free fatty acid levels, which are known regulators of leptin secretion. In addition, C/EBP α, PPAR γ, or SREBP1a protein levels did not change with VPA treatment, suggesting the levels of these transcription factors are not responsible for the effect of VPA on leptin expression. Evidence from experiments using actinomycin D (ActD) or cyclohexamide (CHX) show that VPA does not promote degradation of leptin mRNA; however, VPA can alter leptin transcription through an unknown mechanism independent of new protein synthesis. These results show that VPA can
have direct effects on adiposities that may contribute to altered energy balance in patients treated with VPA.

### 4.8 ANTI-CANCER ACTIVITY OF VALPROIC ACID

The short chain fatty acid valproic acid (VPA, 2-propylpetanoic acid) is approved for the treatment of epilepsia, bipolar disorders and migraine and clinically used for schizophrenia. In 1999, the first clinical anti-cancer trial using VPA was initiated. Currently, VPA is examined in numerous clinical trials for different leukemia’s and solid tumor entities. In addition to clinical assessment, the experimental examination of VPA as anti-cancer drug is ongoing and many questions remain unanswered. Although other mechanisms may also contribute to VPA-induced anti-cancer effects, inhibition of histone deacetylases appears to play a central role.

### 4.9 THE SECOND GENERATION TO VALPROIC ACID (VPA)

Valproic acid, one of the established AEDs (Anti epilepsy drugs), is in animal models the least potent of the major AEDs. However, due to its wide spectrum of antiepileptic activity, VPA is the most prescribed AED. 33,34 Valproic acid is also an effective (and FDA-approved) drug in migraine prophylaxis and in the treatment of bipolar disorder.

Valproic acid is a simple molecule (isoocatanoic acid) 35 and, thus, a useful, cheap, and readily available starting material for synthesizing an array of derivatives that can become CNS-active follow-up compounds to VPA. We believe that novel chemical modifications and further development of specific VPA analogues and derivatives will show promising potential in the areas of epilepsy, pain, bipolar disorder, and other related neurological diseases. As VPA is the least potent among the established AEDs, it is possible to develop VPA analogues that will be significantly more potent than the parent compound and will also be nonteratogenic.

There are numerous reports defining the strict structural requirements for the teratogenicity of VPA and its structurally related compounds. 36 Structure-activity relationship studies conducted in mice strains prone to VPA-associated teratogenicity indicate that to be teratogenic, and to cause neural tube defects in mouse embryos, VPA analogues and derivatives should contain a tertiary carbon bound to a carboxylic...
Chapter-4

Novel Valproic acid derivatives

group, a hydrogen atom, and two alkyl chains. A VPA derivative lacking any one of these structural requirements has the potential to become a nonteratogenic entity.\(^{37}\)

For example, the corresponding CNS-active amide of VPA valpmide (VPD) that has a carboxamide moiety instead of a carboxylic group is not teratogenic. Similarly, the active metabolite of VPA is 2-ene-VPA, which does not have an \(\alpha\)-hydrogen to the carboxylic moiety, is also nonteratogenic.\(^{38}\) Valpromide and 2-ene-VPA are potent anticonvulsant compounds that may represent a novel type of second-generation VPA drug. However, as their fraction metabolized to VPA in humans is greater than 90% (for VPD) and approximately 20% (for 2-ene-VPA), their lack of teratogenicity does not offer a clinical advantage over VPA.

Unlike teratogenicity, the current thinking on VPA induced hepatotoxicity (microvesicular steatosis) is that it is not caused by the parent compound but primarily by VPA metabolite(s) with a terminal double-bond: 4-ene-VPA and 2,4-diene-VPA. These metabolites are further biotransformed to chemically reactive intermediates that bind to cellular macromolecules and enzymes involved in the metabolism of fatty acids. The first step in this cascade is the formation of an acyl-coenzyme A (CoA) thioester leading to depletion of CoA in the liver and, consequently, to hepatotoxicity.\(^{39,42}\) Designing substituted aliphatic and alicyclic VPA analogues and \(\alpha\) and \(\beta\) substituted VPA derivatives (amides) to block the formation of these two metabolites should prevent, or at least minimize, the VPA-induced hepatotoxicity.\(^{43,45}\)

Structure–activity relationship studies mapped the structural elements of the VPA molecules responsible for the anticonvulsant activity.\(^{46}\) Subsequent studies showed that constitutional isomers of VPA, such as valnoctic acid (VCA), propylisopropyl acetic acid (PIA), or diisopropylacetic acid (DIA), were less active as anticonvulsants than VPA. However, their respective corresponding amides, propylisopropylacetamide (PID) and diisopropylacetamide (DID), are more potent than VPA. Unlike valproyl esters, VPA amide derivatives act as drugs on their own and not as prodrugs to their corresponding acids.\(^{47}\) Recent SAR data indicate that a pharmacokinetic-based design is an attractive and feasible approach for the development of nonteratogenic and nonhepatotoxic CNS-active second generation to VPA drugs.
4.10 AIM OF CURRENT WORK

Over and above, the known antiepileptic properties of valproic acids and its salt, renewed interest of these molecules in anticancer\(^1\) and antiviral\(^2\) therapy has led wide interest in newer derivatives of these molecules.

Currently, the leading compounds that are second generation to VPA can be divided into three groups (Figs. 1, 2, and 3) 1) Alkyl analogues of VPA and their amide derivatives, including chiral and achiral constitutional isomers of VPD (Fig. 1) 2) Amide derivatives of TMCA, a cyclopropyl analogue of VPA (FIG. 2) 3) Conjugation products between VPA and neuroinhibitory amino acids: GABA, glycine, taurine, and their corresponding amides (Fig. 3). Some valproic acid drugs & derivatives under preclinical/phase clinical trials.\(^{48,49}\)

FIG. 1. Valproic acid (VPA), its cyclopropyl analogue 2,2,3,3-tetramethylcyclopropanecarboxylic acid (TMCA) and their corresponding amides valpromide (VPD) and 2,2,3,3-tetramethylcyclopropanecarboxamide (TMCD).

FIG. 2. Chemical structures of CNS-active amides of valproic acid (VPA) analogues with the potential to become second-generation VPA drugs. Valnoctamide (VCD), propylisopropylacetamide (PID), di-isopropylacetamide (DID), N-methyl-2,2,3,3-tetramethylcyclopropanecarboxamide (MTMCD), 2,2,3,3-tetramethylcyclopropylcarboxyl urea (TMC-urea), N-methoxy-2,2,3,3-tetramethylcyclopropylcarbonylurea (OM-TMCD), and isovaleramide (NPS 1776). * Indicates the chiral center.

FIG. 3. Valrocemide and conjugation products between valproic acid and neuroinhibitory amino acids and their corresponding amides.

In the current chapter, the valproic ‘Core’ structure was used as a starting material to synthesize valproic acid containing thiazole derivatives. The reaction of valproate with hydrazine hydrates and followed by chloroacetyl chloride and thiourea afford the desired substituted thiazole derivatives.

4.11 REACTION SCHEME

a) CH$_3$OH, gla.CH$_3$COOH, 60-70 °C
b) NH$_2$-NH$_2$, 110 °C
c) DMF, TEA, ClCH$_2$COCl, 0-5 °C
d) (i) Thiourea, CH$_3$OH, 65 °C
   (ii) Thiourea derivatives, CH$_3$OH, 65 °C
e) DMF, TEA, Substituted acid chloride, 0-5 °C
4.12 REACTION MECHANISM

**Step-1**

**Step-2**

**Step-3**

**Step-4**
4.13 EXPERIMENTAL

❖ Preparation of Methyl 2-propyl pentanoate

2-Propyl pentanoic acid (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of methanol was added into above flask. 3-4 drops of Con. sulphuric acid was added as a catalyst. The reaction mixture was refluxed for 12-14 h on water bath. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4: 6) as a mobile phase. After the reaction was completed, excess of methanol was removed under reduced pressure. The separated product was extracted using ethyl acetate (30 ml × 3), the combined organic layer was washed using 5% sodium bicarbonate solution (20 ml × 2) followed by water (20 ml × 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product in a viscous liquid form. Yield - 90 %, B. P. - 170-172 °C.¹


❖ Preparation of 2-Propyl pentanohydrazide

Methyl 2-propylpentanoate (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of hydrazine hydrate was added into above flask. The reaction mixture was refluxed on water bath for 12-14 h. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4: 6) as a mobile phase. After the reaction to be completed, the mixture was cooled to room temperature to give 2-propylpentanohydrazide as a white colored shining fluffy product. Yield - 60 %, M. P. - 124-126 °C.²

² Benoit-Guyod, L. Jean; Chemica Therapeutica 1968, 3(5), 336-42.

❖ Preparation of N’-(2-chloroacetyl)-2-propyl pentanehydrazide

2-Propylpentanohydrazide (0.01 mole) was charged in 10 ml of tetrahydrofuran into 250 ml round bottom flask. Then add triethylamine (0.015 mole) and chloroacetyl chloride (0.01 mole) at 0-5 °C. The reaction mixture was stirred at room temperature overnight. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene: ethyl acetate (4: 6) as a mobile phase.
After completion of the reaction, the reaction mixture was poured into crushed ice. \(N^\prime\)-(2-chloroacetyl)-2-propylpentanethyldrazide a brown colored solid product.

Note: The entire reaction was carried out under nitrogen atmosphere.

- **General procedure of \(N^\prime\)-(substituted 2-aminothiazol-4-yl)-2-propylpentanethyldrazide**

\(N^\prime\)-(2-chloroacetyl)-2-propyl pentanethyldrazide (0.01 mole) was charged into 250 ml round bottom flask. 10 ml of methanol was added to dissolve it. Then add 0.015 mole of substituted thiourea. Resulting reaction mixture was reflux at 2-3 h. The progress and the completion of the reaction were checked by silica gel-G \(F_{254}\) thin layer chromatography using toluene: ethyl acetate (3:7) as a mobile phase. After the reaction was complete the mixture was poured into crushed ice to give white solid compound.

- **General procedure of \(N^\prime\)-(2-(substituted benzamido)thiazol-4-yl)-2-propyl-pentanethyldrazide**

\(N^\prime\)-(2-aminothiazol-4-yl)-2-propylpentanethyldrazide (0.01 mole) was charged into 250 ml round bottom flask. 10 ml of tetrahydrofuran was added to dissolve it. Add 0.015 mole of triethylamine as a catalyst then added 0.01 mole of substituted acid chlorides at 0-5 °C. The reaction mixture was stirred at RT overnight. The progress and the completion of the reaction were checked by silica gel-G \(F_{254}\) thin layer chromatography using toluene: ethyl acetate (3: 7) as a mobile phase. After the reaction was complete mixture poured into crushed ice. \(N^\prime\)-(2-(substituted benzamido)thiazol-4-yl)-2-propylpentanethyldrazide a brown colored solid product.

Note: The entire reaction was carried out under nitrogen atmosphere.
### 4.14 PHYSICAL DATA

#### TABLE: 1 PHYSICAL DATA OF \( N'-(2-\text{(SUBSTITUTED BENZAMIDO)} \)<br>THIAZOL-4-YL)-2-PROPYLPENTANEHYDRAZIDE<br>DERIVATIVES

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Substituted</th>
<th>M.F.</th>
<th>M. P (°C)</th>
<th>Rf value</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPB-67</td>
<td></td>
<td>( C_{11}H_{20}N_{4}OS )</td>
<td>150-152</td>
<td>0.32</td>
<td>81</td>
</tr>
<tr>
<td>DPB-68</td>
<td></td>
<td>( C_{12}H_{22}N_{4}OS )</td>
<td>163-165</td>
<td>0.38</td>
<td>75</td>
</tr>
<tr>
<td>DPB-69</td>
<td></td>
<td>( C_{13}H_{22}N_{4}O_{2}S )</td>
<td>142-146</td>
<td>0.36</td>
<td>72</td>
</tr>
<tr>
<td>DPB-70</td>
<td></td>
<td>( C_{11}H_{21}N_{5}OS )</td>
<td>182-184</td>
<td>0.28</td>
<td>62</td>
</tr>
<tr>
<td>DPB-71</td>
<td></td>
<td>( C_{23}H_{28}N_{4}OS )</td>
<td>225-227</td>
<td>0.42</td>
<td>69</td>
</tr>
<tr>
<td>DPB-72</td>
<td></td>
<td>( C_{18}H_{24}N_{4}O_{2}S )</td>
<td>189-191</td>
<td>0.38</td>
<td>72</td>
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<tr>
<td>DPB-73</td>
<td></td>
<td>( C_{19}H_{26}N_{4}O_{2}S )</td>
<td>195-197</td>
<td>0.35</td>
<td>67</td>
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<tr>
<td>DPB-74</td>
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<td>( C_{19}H_{26}N_{4}O_{2}S )</td>
<td>193-195</td>
<td>0.36</td>
<td>69</td>
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<tr>
<td>DPB-75</td>
<td></td>
<td>( C_{19}H_{26}N_{4}O_{2}S )</td>
<td>197-199</td>
<td>0.35</td>
<td>73</td>
</tr>
<tr>
<td>DPB-76</td>
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<td>( C_{18}H_{23}ClN_{4}O_{2}S )</td>
<td>183-185</td>
<td>0.41</td>
<td>79</td>
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<tr>
<td>DPB-77</td>
<td></td>
<td>( C_{18}H_{23}ClN_{4}O_{2}S )</td>
<td>180-182</td>
<td>0.39</td>
<td>75</td>
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<tr>
<td>DPB-78</td>
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<td>( C_{18}H_{23}ClN_{4}O_{2}S )</td>
<td>186-188</td>
<td>0.42</td>
<td>76</td>
</tr>
<tr>
<td>DPB-79</td>
<td></td>
<td>( C_{18}H_{23}N_{5}O_{4}S )</td>
<td>175-177</td>
<td>0.39</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Structure</td>
<td>Formula</td>
<td>M.p. (°C)</td>
<td>Rf</td>
<td>Yield (%)</td>
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<td>DPB-80</td>
<td><img src="image1.png" alt="Image" /></td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;S</td>
<td>179-181</td>
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<td>63</td>
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<td><img src="image2.png" alt="Image" /></td>
<td>C&lt;sub&gt;19&lt;/sub&gt;H&lt;sub&gt;26&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>196-198</td>
<td>0.35</td>
<td>70</td>
</tr>
</tbody>
</table>

R<sub>f</sub> value was calculated using solvent system, Toluene: Ethyl Acetate (3: 7).
4.15 SPECTRAL STUDY

- **IR spectra**

Infra Red spectra were taken on SHIMADZU FTIR-435 spectrometer using KBr pellet method. The characteristic carbonyl group of –CONH in valporic acid moiety was observed at 1690-1630 cm\(^{-1}\). Amine (>NH) observed a broad peak between 3200-3000 cm\(^{-1}\). Methylene gp (>CH\(_2\)) observed at 3000-2850 cm\(^{-1}\). Methyl (-CH\(_3\)) observed at 1350 cm\(^{-1}\). DPB- 68 and DPB-69 of IR spectra are given on page no: - 138 and 139.

- **\(^1\)H NMR spectra**

\(^1\)H NMR spectra were recorded on a Bruker AC 400 MHz FT-NMR spectrometer using TMS (Tetramethyl Silane) as an internal standard and DMSO-d\(_6\) & CDCl\(_3\) as a solvent. In the NMR spectra of \(N'-(2-(\text{substituted benzamido}) \text{ thiazol-4-yl})-2\)-propylpentanehydrazide various proton values of methylene (-CH\(_2\)), amine (-NH) and methyl (-CH\(_3\)) etc. were observed as under.

The values for methyl (-CH\(_3\)) proton is observed between 0.8-1.3 \(\delta\) ppm. The values for methylene (-CH\(_2\)) proton is observed between 1.3-2.2 \(\delta\) ppm. The -NH protons of amide group (>CONH) at 7.0-11.0 \(\delta\) ppm. The signal due to NH proton of amide group was observed at 10.0-10.5 \(\delta\) ppm value. DPB-67 and DPB-69 of \(^1\)H NMR spectra are given on page no: - 135 to 138.

1. The proton no.11 of amine group gave a characteristic broad singlet at 5.38 \(\delta\) ppm.

2. The proton no.10 of thiazole ring gave a characteristic singlet at 3.88 \(\delta\) ppm.

3. Proton no. 1and 4 of propyl chain of two methyl group of six proton gave a multiplet at 0.85 \(\delta\) ppm - 0.89 \(\delta\) ppm.
4. Proton no. 2, 3, 5 and 6 of di-propyl chain gave a multiplet at 1.25 δ ppm - 1.38 δ ppm. It showed expanded spectra.

5. Proton no. 7 of di-propyl chain gave a multiplet at 2.27 δ ppm-2.32 δ ppm.

6. Two most deshielded proton no.8 and 9 of secondary amine in hydrazide linkage of two (-NH) group gave two separable singlet in the down field at 7.91 δ ppm and 10.02 δ ppm respectively.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton, the proposed structure for compound DPB-67 was confirmed.

\[ \text{13C NMR spectra} \]

\[ \text{13C NMR spectra were recorded on a Bruker AC 400 MHz FT-NMR spectrometer using DMSO-d}_6 \text{ & CDCl}_3 \text{ as a solvent. In the 13C NMR spectra of } N'-(2-((substituted benzamido)thiazol-4-yl))-2-propylpentanehydrazide \text{ various carbon values of methylene (-CH}_2\text{), keto (>C}=O\text{), methyl (-CH}_3\text{) and aromatic carban (Ar-H) etc. were observed as under. The values for methylene (-CH}_2\text{) carban is observed between } \delta 35-65 \text{ ppm. The }>C=O \text{ carban observed at 160-180 } \delta \text{ ppm. Aromatic carbon shows between 110-140 } \delta \text{ ppm. DPB-67 and DPB-69 of 13C NMR spectra are given on page no: - 135 to 138.} \]

1. The carbon no. 1 and 4 methyl group, appear at 13.7 δ ppm it shows in spectra.
2. The carbon no. 2 and 5 methylene group, appear at 20.12 δ ppm.
3. The carbon no. 3 and 6 methylene group, appear at 34.8 δ ppm.
4. The carbon no. 7 appears at 43.55 δ ppm it shows in spectra.
5. The carbon no. 8 appears at 176.6 δ ppm due to the effect of carbonyl group.
6. The carbon no. 9 and 11 thiazole ring appear at 173.1 δ ppm due to the effect of nitrogen atom.
7. The carbon no. 10 thiazole ring appears at 99.49 δ ppm.
Mass spectra

The mass spectrum of compounds were recorded by Shimadzu GC-MS-QP-2010 spectrometer (EI method). The mass spectrum of compounds was obtained by positive chemical ionization mass spectrometry. The molecular ion peak and the base peak in all compounds were clearly obtained in mass spectral study. The molecular ion peak (M^+) values are in good agreement with molecular formula of all the compounds synthesized. DPB- 67, DPB-68 and DPB-69 of Mass spectra are given on page no.- 134 and 135.

Elemental analysis

Elemental analysis of the synthesized compounds was carried out on Vario EL Carlo Erba 1108 model at Saurashtra University, Rajkot which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The elemental analysis data are given for individual compounds.
4.16 SPECTRAL CHARACTERIZATION

N’-(2-aminothiazol-4-yl)-2-propylpentanehydrazide (DPB-67)

IR (KBr) cm⁻¹: 3466 (N-H str), 2956 (-CH₃ str.), 2931 (-CH₂ str.), 2874 (-CH₃ str.), 1640 (-CONH), 1608 (N-H bending), 1452 (-CH₃ ben), 1367 (-CH₂ ben). ¹H NMR 400 MHz: (DMSO-d₆, δ ppm): 0.89 (m, 6H, -CH₃), 1.32 (m, 6H, -CH₂), 1.57 (m, 2H, -CH₂), 2.29 (m, 1H, -CH), 3.88 (s, 1H, -CH), 5.38 (s, broad, 2H,-NH₂), 7.91 (s, broad, 1H,-NH), 10.02 (s, broad, 1H,-NH). ¹³C NMR 400 MHz: (DMSO-d₆, δ ppm): 13.78, 20.12, 34.85, 28.70, 43.86, 99.49, 173.1, 176.6 Mass: [m/z (%)], M. Wt.: 256. Elemental analysis, Calculated: C, 51.53; H, 7.86; N, 21.85 Found: C, 51.59; H, 7.79; N, 21.70

N’-(2-(methylamino)thiazol-4-yl)-2-propylpentanehydrazide (DPB-68)

IR (KBr) cm⁻¹: 3408 (N-H str), 2924 (-CH₃ str.), 287 (-CH₂ str.), 2818 (-CH₃ str.), 1653 (-CONH), 1602 (N-H bending), 1481 (-CH₃ ben), 1369 (-CH₂ ben). Mass: [m/z (%)], M. Wt.: 270. Elemental analysis, Calculated: C, 53.30; H, 8.20; N, 20.72 Found: C, 53.32; H, 8.28; N, 20.65

N’-(2-acetamidothiazol-4-yl)-2-propylpentanehydrazide (DPB-69)

IR (KBr) cm⁻¹: 3489 (N-H str), 2963 (-CH₃ str.), 2870 (-CH₂ str.), 2775 (-CH₃ str.), 1654 (-CONH), 1631 (N-H bending), 1447 (-CH₃ ben), 1367 (-CH₂ ben). ¹H NMR 400 MHz: (DMSO-d₆, δ ppm): 0.89 (m, 6H, -CH₃), 1.33 (m, 6H, -CH₂), 1.61 (m, 2H, -CH₂), 2.32 (m, 1H, -CH), 3.20 (s, 3H, -CH₃), 3.86 (s, 1H, -CH), 7.78 (s, broad, 1H,-NH), 9.93 (s, broad, 1H,-NH), 11.66 (s, broad, 1H,-NH). ¹³C NMR 400 MHz: (DMSO-d₆, δ ppm): 13.70, 20.15, 34.56, 43.76, 173.2 Mass: [m/z (%)], M. Wt.: 298. Elemental analysis, Calculated: C, 52.32; H, 7.43; N, 18.78 Found: C, 52.22; H, 7.49; N, 18.85

N’-(2-hydrazinylthiazol-4-yl)-2-propylpentanehydrazide (DPB-70)

IR (KBr) cm⁻¹: 3455 (N-H str), 2947 (-CH₃ str.), 2952 (-CH₂ str.), 2849 (-CH₃ str.), 1641 (-CONH), 1625 (N-H bending), 1452 (-CH₃ ben), 1375 (-CH₂ ben). Mass: [m/z (%)], M. Wt.: 271. Elemental analysis, Calculated: C, 48.68; H, 7.80; N, 25.81 Found: C, 48.65; H, 7.77; N, 25.89

N’-(2-(diphenylamino)thiazol-4-yl)-2-propylpentanehydrazide (DPB-71)

IR (KBr) cm⁻¹: 3445 (N-H str), 2951 (-CH₃ str.), 2945 (-CH₂ str.), 2852 (-CH₃ str.), 1658 (-CONH), 1630 (N-H bending), 1458 (-CH₃ ben), 1371 (-CH₂ ben), 3011 (=C-
N’-(2-(benzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-72)

IR (KBr) \(\text{cm}^{-1}\): 3440 (N-H str), 2959 (-CH\(_3\) str.), 2947 (-CH\(_2\) str.), 2859 (-CH\(_3\) str.), 1648 (-CONH), 1636 (N-H bending), 1457 (-CH\(_3\) ben), 1372 (-CH\(_2\) ben), 3018 (=C-H, str), 3055 (Ar, C-H, str), 1535 (Ar, C=C, str). Mass: [m/z (%)], M. Wt.: 408.

Elemental analysis, Calculated: C, 67.61; H, 6.91; N, 13.71 Found: C, 67.66; H, 6.85; N, 13.68

N’-(2-(4-methylbenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-73)

IR (KBr) \(\text{cm}^{-1}\): 3441 (N-H str), 2962 (-CH\(_3\) str.), 2941 (-CH\(_2\) str.), 2862 (-CH\(_3\) str.), 1642 (-CONH), 1639 (N-H bending), 1462 (-CH\(_3\) ben), 1370 (-CH\(_2\) ben), 3022 (=C-H, str), 3053 (Ar, C-H, str), 1545 (Ar, C=C, str). Mass: [m/z (%)], M. Wt.: 360.

Elemental analysis, Calculated: C, 60.97; H, 7.01; N, 15.54 Found: C, 60.95; H, 7.03; N, 15.50

N’-(2-(3-methylbenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-74)

IR (KBr) \(\text{cm}^{-1}\): 3439 (N-H str), 2955 (-CH\(_3\) str.), 2953 (-CH\(_2\) str.), 2849 (-CH\(_3\) str.), 1649 (-CONH), 1645 (N-H bending), 1478 (-CH\(_3\) ben), 1375 (-CH\(_2\) ben), 3049 (Ar, C-H, str), 1549 (Ar, C=C, str). Mass: [m/z (%)], M. Wt.: 374.

Elemental analysis, Calculated: C, 60.94; H, 7.00; N, 14.96 Found: C, 60.98; H, 7.04; N, 14.92

N’-(2-(2-methylbenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-75)

IR (KBr) \(\text{cm}^{-1}\): 3446 (N-H str), 2956 (-CH\(_3\) str.), 2951 (-CH\(_2\) str.), 2855 (-CH\(_3\) str.), 1647 (-CONH), 1641 (N-H bending), 1470 (-CH\(_3\) ben), 1369 (-CH\(_2\) ben), 3050 (Ar, C-H, str), 1552 (Ar, C=C, str). Mass: [m/z (%)], M. Wt.: 374.

Elemental analysis, Calculated: C, 60.94; H, 7.00; N, 14.96 Found: C, 60.95; H, 7.05; N, 14.98

N’-(2-(4-chlorobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-76)

IR (KBr) \(\text{cm}^{-1}\): 3442 (N-H str), 2958 (-CH\(_3\) str.), 2956 (-CH\(_2\) str.), 2849 (-CH\(_3\) str.), 1648 (-CONH), 1647 (N-H bending), 1465 (-CH\(_3\) ben), 1371 (-CH\(_2\) ben), 3016 (=C-H, str), 3051 (Ar, C-H, str), 1549 (Ar, C=C, str), 742 (C-Cl). Mass: [m/z (%)], M.
Wt.: 394. Elemental analysis, Calculated: C, 54.74; H, 5.87; N, 14.19 Found: C, 54.76; H, 5.89; N, 14.22

**N’-(2-(3-chlorobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-77)**

IR (KBr) cm$^{-1}$: 3441 (N-H str), 2959 (-CH$_3$ str.), 2957 (-CH$_2$ str.), 2852 (-CH$_3$ str.), 1656 (-CONH), 1650 (N-H bending), 1462 (-CH$_3$ ben), 1378 (-CH$_2$ ben), 3010 (=C-H, str), 3056 (Ar, C-H, str), 1552 (Ar, C=C, str), 748 (C-Cl). Mass: [m/z (%)], M.

Wt.: 394. Elemental analysis, Calculated: C, 54.74; H, 5.87; N, 14.19 Found: C, 54.70; H, 5.83; N, 14.20

**N’-(2-(2-chlorobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-78)**

IR (KBr) cm$^{-1}$: 3448 (N-H str), 2958 (-CH$_3$ str.), 2953 (-CH$_2$ str.), 2848 (-CH$_3$ str.), 1648 (-CONH), 1644 (N-H bending), 1460 (-CH$_3$ ben), 1376 (-CH$_2$ ben), 3014 (=C-H, str), 3047 (Ar, C-H, str), 1549 (Ar, C=C, str), 747 (C-Cl). Mass: [m/z (%)], M.

Wt.: 394. Elemental analysis, Calculated: C, 54.74; H, 5.87; N, 14.19 Found: C, 54.69; H, 5.84; N, 14.23

**N’-(2-(4-nitrobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-79)**

IR (KBr) cm$^{-1}$: 3452 (N-H str), 2955 (-CH$_3$ str.), 2954 (-CH$_2$ str.), 2847 (-CH$_3$ str.), 1648 (-CONH), 1640 (N-H bending), 1463 (-CH$_3$ ben), 1374 (-CH$_2$ ben), 3025 (=C-H, str), 3047 (Ar, C-H, str), 1556 (Ar, C=C, str). Mass: [m/z (%)], M. Wt.: 405.

Elemental analysis, Calculated: C, 53.32; H, 5.72; N, 17.27 Found: C, 53.35; H, 5.70; N, 17.30

**N’-(2-(3-nitrobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-80)**

IR (KBr) cm$^{-1}$: 3442 (N-H str), 2958 (-CH$_3$ str.), 2956 (-CH$_2$ str.), 2847 (-CH$_3$ str.), 1652 (-CONH), 1647 (N-H bending), 1461 (-CH$_3$ ben), 1370 (-CH$_2$ ben), 3016 (=C-H, str), 3059 (Ar, C-H, str), 1549 (Ar, C=C, str). Mass: [m/z (%)], M. Wt.: 405.

Elemental analysis, Calculated: C, 53.32; H, 5.72; N, 17.27 Found: C, 53.30; H, 5.71; N, 17.29

**N’-(2-(2-phenylacetamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-81)**

IR (KBr) cm$^{-1}$: 3452 (N-H str), 2955 (-CH$_3$ str.), 2951 (-CH$_2$ str.), 2856 (-CH$_3$ str.), 1640 (-CONH), 1644 (N-H bending), 1461 (-CH$_3$ ben), 1375 (-CH$_2$ ben), 3022 (=C-H, str), 3045 (Ar, C-H, str), 1549 (Ar, C=C, str). Mass: [m/z (%)], M. Wt.: 374.
Elemental analysis, Calculated: C, 60.94; H, 7.00; N, 14.96 Found: C, 60.95; H, 7.03; N, 14.98

4.17 CONCLUSION

In conclusion, several novel valproate containing thiazole derivatives for biological activity were synthesized. The reaction of valproate with hydrazine hydrate afforded the 2-propyl pentanohydrazide, which on reaction with chloroacetyl chloride yielded the N’-(2-chloroacetyl)-2-propyl pentanehydrazide. The desired thiazole derivatives had been synthesized by the reaction of thiourea with N’-(2-chloroacetyl)-2-propyl pentanehydrazide. All the newly synthesized compounds were well characterized by spectroscopy techniques.
4.18 REPRESENTATIVE SPECTRUM

Mass spectrum of $N'$-(2-aminothiazol-4-yl)-2-propylpentanehydrazide (DPB-67)

Mass spectrum of $N'$-(2-(methylamino)thiazol-4-yl)-2-propylpentanehydrazide (DPB-68)
Mass spectrum of \( \text{N}'-(2-(4-nitrobenzamido)thiazol-4-yl)-2-propylpentanehydrazide (DPB-79) \)

\[ \text{Mol. Wt.: 405.47} \]

\( ^1\text{H NMR spectrum of N}'-(2-aminothiazol-4-yl)-2-propylpentanehydrazide (DPB-67) \)
$^{13}$C NMR spectrum of $N'-(2$-aminothiazol-4-yl)-2-propylpentanehydrazide (DPB-67)
\(^1\)H NMR spectrum of \(N^\prime\)-(2-acetamidothiazol-4-yl)-2-propylpentanedihydrazide (DPB-69)
$^{13}$C NMR spectrum of $N'$-(2-acetamidothiazol-4-yl)-2-propylpentanehydrazide (DPB-69)

IR spectrum of $N'$-(2-acetamidothiazol-4-yl)-2-propylpentanehydrazide (DPB-69)
IR spectrum of \( N'(2-\text{methylamino})\text{thiazol-4-yl})-2\text{-propylpentanehydrazide} \) (DPB-68)
4.19 REFERENCE

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