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DEVELOPMENT OF FIVE-MEMBER HETEROCYCLIC COMPOUNDS AS POTENTIAL NAV 1.7 INHIBITORS

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ABSTRACT

Pain is often defined as a single form of the nervous system for abnormal things in the human body. Neuropathic pain (NP) is a kind of pain that is a worry of nerve damage and usually, it is a chronic type. Sodium Channel 1.7 (Nav 1.7) is an forthcoming biological target against neuropathic pain management. Several heterocyclic compounds are reported for potentials against neuropathic pain. 10 different heterocyclic derivatives from thiadiazole class were synthesized,virtually analyzed against Nav 1.7 for inhibition potential and carry out biological activity by Streptozotocine (STZ) induced diabetic pain model. The developed thiadiazole were confirmed via spectral analysis and showed excellent binding ability with Nav 1.7, which can be further sightseen for biological activity.

Keywords: Pain, Neuropathic pain, Thiadiazole, Docking, STZ

INTRODUCTION:

Heterocyclic compounds are compounds with one or more heteroatoms in their structure. Heterocyclic compounds are known for their biological potentials. Heterocyclic systems like Thiadiazole **[1-3]** are known for their biological activities. Numerous activities like antimicrobial, anticancer, antitubercular, anti-inflammatory, and analgesic are shown by heterocyclic compounds **[4, 5]**. Neuropathic pain is one of the types of pain which involves nerve damages or injury to the nerve ending. This type of pain is often chronic one which can be caused by

alcoholism, diabetes, AIDS, and chronic nerve disorders **[1-6]**. Neuropathic pain is often treated with antiseizure drugs like Gabapentin, Pregabalin, Topiramate, Carbamazepine, and certain antidepressant agents like Amitriptyline, Nortriptyline, and Venlafaxine **[6]**. The development of selective and specific molecules targeting neuropathic pain needs time. Sodium Channel 1.7 (Nav 1.7) is one of the upcoming targets for the development of molecules against neuropathic pain. Nav1.7 is a channel that is encoded by the SCN9A gene normally it is found to be present in the high levels in nociceptive (pain) neurons in the dorsal root ganglion and trigeminal ganglion and sympathetic ganglion neurons which is a component of the ANS. Sodium channel NaV1.7 plays an important role in the maintenance of the threshold for the action potential in the primary sensory neurons, due to this reason Nav 1.7 is the main research area for many researchers **[7, 8]**. Molecular docking is one of the key techniques utilized for molecular scrutiny before the synthesis. Docking analysis is also called the molecular interaction analysis in which the interaction between the macromolecule and macromolecule is analyzed. The docking analysis can give an insight into the molecular behavior in the biological system and which will be helpful to analyze the toxicity, metabolism, and off-target effects associated with the designed set of molecules. In this research paper, we are reporting the synthesis of the 10 different heterocyclic molecules and their biological activity of active scaffold by streptozotine induced neuropathic pain model **[9]**.

EXPERIMENTAL

Synthesis: (Figure 1, Table 1)

Table 1: Substituent's in synthesized derivatives

Sr. No	Compound Code	R-Group	Molecular formula
01	SS2 (4)	H	$C_{12}H_{12}N_6S$
02	SS2 (5)	$-C_6H_5$	$C_{18}H_{16}N_6S$
03	SS2 (6)	$-CH_2-COOH$	$C_{14}H_{14}N_6O_2S$
04	SS2 (7)	$2-C_6H_5-Cl$	$C_{18}H_{15}ClN_6S$
05	SS2 (8)	$4-C_6H_5-COOH$	$C_{19}H_{16}N_6O_2S$
06	SS2 (9)	CH_3	$C_{13}H_{14}N_6S$
07	SS2 (10)	$-CH_2-CH_3$	$C_{14}H_{16}N_6S$
08	SS2 (11)	$-CO-CH_3$	$C_{14}H_{14}N_6OS$
09	SS2 (12)	$-CH-(CH_3)_2$	$C_{15}H_{18}N_6S$
10	SS2 (13)	$CH_3-CH-C_2H_5$	$C_{16}H_{20}N_6S$

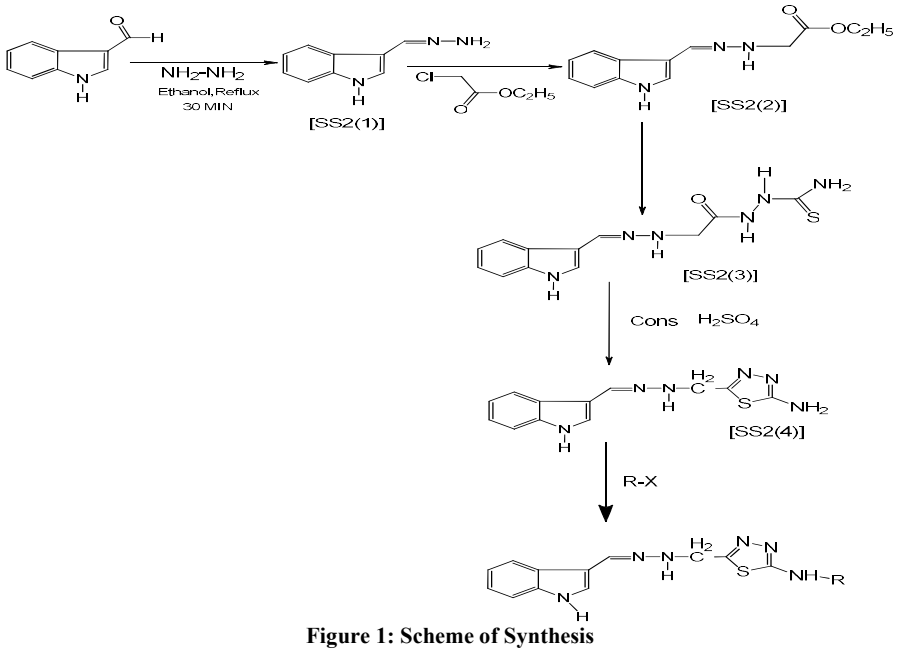

Figure 1: Scheme of Synthesis

Synthesis of 3-(Hydrazonomethyl)-1H-indole :- SS2(1)

The equimolar concentration of Indole-3-carboxaldehyde (0.01M) and hydrazine sulfate (0.01M) were refluxed in 25 ml ethanol for 6 hours in a water bath. After completion of the reaction, the mixture was poured into crushed ice and recrystallized by using ethanol. Melting point -1640C, Rf Value-0.58.Percentage yield:-62.93.

Synthesis of 1-(2-(1H-indol-3yl)methylene)hydrazinyl)butan-2one(II):- SS2(2)

A mixture of Synthesis of 3-(Hydrazonomethyl)-1H-indole (II) (0.01M), 0.01M chloroethyl acetate, 0.01 M potassium acetate, 23- drops of DMA in 25 ml of acetone taken in a round bottom flask, and the mixture refluxed for 28 hrs and the resulting solution poured in crushed ice. Filter the crude product and recrystallize it in ethanol. Melting point-183^{0}c.R^f value-0.52

Synthesis of 2-(2-(2- ((1 H – indol - yl) methylene) hydrazinyl) acetyl) hydrazine carbothioamide (III):-SS2(3)

An equimolar mixture of 1-(2-(1H-indol-3yl)methylene)hydrazinyl)butan-2-one , and thiosemicarbazone was refluxed with ethanol for 26 hrs. After completion of the reaction, the mixture was poured into crushed ice. Filter the crude product and recrystallize it in ethanol. Melting point-2340c.Rf value-0.58

Synthesis of 5-((2-(1H indol-3yl)methylene)hydrazinyl)methyl-1,3,4-thiadiazol-2-amine:-SS2(4)

A mixture of 5-((2-(1H indol-3yl)methylene)hydrazinyl)methyl-1,3,4-thiadiazol-2-amine(0.01M), 0.01M sodium

hydroxide in 25 ml ethanol refluxed for 24 hrs . After completion of the reaction, the mixture acidifies with conc. sulphuric acid and mixture poured in crushed ice. Recrystallized crude product in ethanol.Melting point-2100c.Rf value-0.48

Synthesis of 5-((2-(1H indol-3yl)methylene)hydrazinyl)methyl)-N-alkyl-1,3,4-thiadiazol-2-amine:-Thiadiazole Derivative

A mixture of 5-((2-(1H indol-3yl)methylene)hydrazinyl)methyl-1,3,4-thiadiazol-2-amine (0.001M),substituted alkyl halide (0.01M), 5ml sodium hydroxide (1%) in 25 ml of iso-propyl alcohol. Reflux the mixture for 25 hours. After completion of reflux add 3-4 drops of dil. Hydrochloric acid. Mixture poured in crushed ice. Filter the crude product and recrystallize it in ethanol.

Molecular Docking [10-12]

Molecular docking was performed to assess the binding ability of the designed derivatives with Sodium Channel NAV 1.7. Structure of the Sodium Channel NAV 1.7(5EK0). was downloaded from the free protein databank www.rcsb.org **[10, 11]** and utilized for docking analysis. Grip-based docking analysis was performed.

Pharmacological activity:

The pharmacological screening of synthesized compounds carried out by strptozotcine (STZ) induced diabetic pain model. After overnight fasting these rats were induced by diabetic condition with STZ (60 mg/kg in sodium citrate buffer solution at pH 4.5). Diabetic state was confirmed after 48-72 hours by determining glucose level from tail vein blood using test strips. Animal having a glucose level more than 250mg/dl in blood where consider for present study. The animals were separated into different groups. Group-I is a diabetic control receive, Group-II is a standard group where Pregabline were given in 10 mg/kg dose ,Group-III SS2(8) to Group-IV SS2(9) contains synthetic molecules with 100mg/kg dos. In all group rat's diabetes was induced by injecting STZ in 60 mg/kg concentration. Biological behaviour of the molecules after each 07 days interval after 28 days sciatic nerve was dissected for further biochemical study.

RESULT AND DISCUSSION

SYNTHESIS:

All the targeted derivatives are synthesized in a very good yield and their physicochemical constants were recorded for the initial confirmation of the synthesis as shown in **Table 2**. Synthesized derivatives were further confirmed via various spectral techniques like IR, NMR, and Mass to confirm the synthesized compounds. The results of the spectral analysis are given below.

Table 2: Physicochemical data of the synthesized derivatives

Sr. No	Compound Code	R-Group	Molecular formula	Molecular Weight	Melting point in C	R^f value
01	SS2 (4)	H	$C_{12}H_{12}N_6S$	272	234	0.57
02	SS2 (5)	$-C_6H_5$	$C_{18}H_{16}N_6S$	348	213	0.46
03	SS2 (6)	$-CH_2-COOH$	$C_{14}H_{14}N_6O_2S$	330	233	0.55
04	SS2 (7)	$2-C_6H_5-Cl$	$C_{18}H_{15}ClN_6S$	382	188	0.56
05	SS2 (8)	$4-C_6H_5-COOH$	$C_{19}H_{16}N_6O_2S$	392	192	0.82
06	SS2 (9)	CH_3	$C_{13}H_{14}N_6S$	286	178	0.85
07	SS2 (10)	$-CH_2-CH_3$	$C_{14}H_{16}N_6S$	300	182	0.68
08	SS2 (11)	$-CO-CH_3$	$C_{14}H_{14}N_6OS$	314	189	0.80
09	SS2 (12)	$-CH-(CH_3)_2$	$C_{15}H_{18}N_6S$	314	195	0.78
10	SS2 (13)	$CH_3-CH-C_2H_5$	$C_{16}H_{20}N_6S$	328	192	0.89

All synthesized compounds are firstly conformed by TLC by Benzene: Ethyl acetate (7:3) solvents as mobile phase and later one compounds characterized by H^1-NMR, C^{13}-NMR, Mass and IR spectroscopy.

Compound- [SS2(2)] : IR (KBr) cm$^-$:3140(NH stretching), 1680.20(C=O Stretching) 1575(aromatic C=C stretching);1611 (C=N stretching); **^{1}H NMR :**([D,]DMSO): δ 1.13(s, 3H,-CH3),3.61(s, 2H,-CH2),4.23(s, 2H,-CH2),7.19(d,2H $-NH_2$), 7.10-8.08(Ar-H), 8.09 (s, 1H,-CH), 8.11 (s, 1H,-CH), 9.94(s, 1H,NH),12.15(s, 1H,NHIndole), **13C** NMR([D,]DMSO): δ = 15.22 (CH3), 54.04(CH2), 61.07(-CH2),112-138 (Ar-C), 158.00(=CH) 184.92 (C=O), **EIMS (M/z) :** Molecular weight Correspond to 246.2 molecular ion peak

Compound-[SS2(3)]: IR (KBr) cm$^-$: 3182.20(NH stretching), 1721(C=O Stretching) 1610(aromatic C=C stretching);1575(aromatic C=N stretching); **^{1}H NMR :**([D,]DMSO): δ 3.58 (s, 1H,-CH2), 7.09-7.40 (q, Ar-H), 7.81 (s, 1H,-CH), 8.02 (s, 1H,-CH), 8.20 (s, 1H,-NH), 8.23 (s, 1H,-NH), 8.29 (s, 1H,-NH), 9.93(s, 1H,NHIndole) **13C** NMR([D,]DMSO): δ58.64(-CH_2),111.10-137.02 (Ar-C),155.04(=CH), 176.52(C=S), 184.92 (C=O) **EIMS (M/z) :** Molecular weight Correspond to 292.1molecular ion peak

Compound- [SS2(4)]: IR (KBr) cm$^-$ 3184(NH stretching), 2916 (C-C aliphatic strretching), 1624(aromatic C=C stretching);1586(aromatic C=N stretching); **^{1}H NMR :**([D,]DMSO): δ 3.58 (s, 1H,-CH2), 7.09-7.40 (q, Ar-H), 7.81 (s, 1H,-CH), 8.02 (s, 1H,-CH), 8.20 (s, 1H,-NH2), 8.23 (s, 1H,-NH), 8.29 (s, 1H,-NH), 9.93(s, 1H,NHIndole) ,**13C** NMR([D,]DMSO): δ60.64(-CH_2),111.91-137.18 (Ar-C), 155.04(=CH), **EIMS (M/z) :** Molecular weight Correspond to 273.9 molecular ion peak

Compound- [SS2(5)]: IR (KBr) cm$^-$ 3100.02 (NH stretching), 2916 (C-C

aliphatic strretching), 1655(aromatic C=C);1573(aromatic C=N stretching); **^{1}H NMR :**([D,]DMSO): δ1.21(s, 2H-CH2)3.69-3.76 (s, 1H,-CH), 7.19-7.92 (q, Ar-H), 8.29 (s, 1H,-NH2), 8.90 (s, 1H,-NH), 9.93(s, 1H,NHIndole), **EIMS (M/z) :** Molecular weight Correspond to 348.2 molecular ion peak.

Compound- [SS2(6)] IR (KBr) cm$^-$ 3109.02 (NH stretching),3000 (-OH stretching) 2916 (C-C aliphatic strretching), 1677(C=O Stretching) 1612(aromatic C=C); 1459(aromatic C=N stretching); **^{1}H NMR :**([D,]DMSO): δ3.93(s, 2H-CH2)4.28(s, 2H-CH2), 7.23-7.46 (q, Ar-H), 7.92 (s, 1H,-NH), 8.90 (s, 1H,-CH), 9.93(s, 1H,NHIndole) 11,76 (s, 1H,-NH2),12,24 (s, 1H,-OH), **EIMS (M/z) :** Molecular weight Correspond to 330 molecular ion peak

Compound- [SS2(7)]: IR (KBr) cm$^-$ 3115 (NH stretching), 2916 (C-C aliphatic strretching) 1612(aromatic C=C); 1456(aromatic C=N stretching);

Compound- [SS2(8)]: IR (KBr) cm$^-$ 3130.30 (NH stretching), 3000(-OH stretching) 2916 (C-C aliphatic strretching), 1704(C=O strretching); 1612(aromatic C=C); 1543(aromatic C=N stretching

Compound- [SS2(9)]: IR (KBr) cm$^-$ 3170 (NH stretching), 2916 (C-C aliphatic strretching) 1606(aromatic C=C); 1420(aromatic C=N stretching)

Compound- [SS2(10)]: IR (KBr) cm$^-$ 3135 (NH stretching), 2916 (C-C aliphatic strretching) 1606(aromatic C=C); 1420(aromatic C=N stretching);

Compound- [SS2(11)]: IR (KBr) cm$^-$ 3219 (NH stretching), 2916 (C-C aliphatic strretching), 1700(C=O strretching); 1616(aromatic C=C); 1543(aromatic C=N stretching

Compound- [SS2(12)]: IR (KBr) cm$^-$ 3209 (NH stretching), 2916 (C-C aliphatic strretching), 1607(aromatic C=C); 1434(aromatic C=N stretching

Compound- [SS2(13)]: IR (KBr) cm$^-$ 3188 (NH stretching), 2916 (C-C aliphatic strretching), 1606(aromatic C=C); 1434(aromatic C=N stretching)

Molecular Docking

Molecular docking was performed to assess the binding ability of the designed derivatives with Sodium Channel NAV 1.7. Structure of the Sodium Channel NAV 1.7. was downloaded from the free protein databank www.rcsb.org and utilized for docking analysis. Grip-based docking analysis was performed. Derivative SS2(4) was found to interact via the formation of hydrogen bond interaction with THR1590 and two charge interactions with ASP1586 GLU1534. Derivative SS2(9)was found showing hydrogen bond interaction with GLU1589and aromatic interactions with TYR1537, TRP1538 charge interaction with GLU1534. SS2(10) was found showing

hydrogen bond interaction with ASP1586 and aromatic interaction with TRP1538 charge interaction with ASP1586. SS2(5)was found showing an aromatic interaction with TYR1537 charge interaction with ASP1586. SS2(11)was found showing hydrogen bond interaction with GLN1530 and charge interaction with ASP1586.SS2(8)was found showing hydrogen bond interaction with GLN1530 and aromatic interactions with TRP1538, PHE1592, charge interaction with ASP1586. SS2(12) was found showing hydrogen bond interaction with GLN1530, aromatic interaction with TRP1538, and charge interaction with ASP1586.SS2(13) was found showing hydrogen bond interaction with GLN1530, aromatic interaction with TYR1537, TRP1538, and charge interaction with GLU1534.SS2(6) was found showing hydrogen bond interaction with GLN1530, charge interaction with ASP1586 alsoSS2(7) was found showing hydrogen bond interaction with GLN1530, charge interaction with ASP1586. All the docking results are shown in **Table 3 and Figure 2-11 [12, 13]**.

Table 3: Docking Interactions of synthesized Molecules

Sr. No	Molecule No	Interactions		
		H bond	Aromatic	Charge
1.	SS2(4)	THR1590		ASP1586, GLU1534
2.	SS2(5)		TYR1537	ASP1586
3.	SS2(6)	GLN1530		ASP1586
4.	SS2(7)	GLN1530		ASP1586
5.	SS2(8)	GLN1530	TRP1538 PHE1592	ASP1586
6.	SS2(09)	GLU1589	TYR1537 TRP1538	GLU1534
7.	SS2(10)	ASP1586	TRP1538	ASP1586
8.	SS2(11)	GLN1530		ASP1586
9.	SS2(12)	GLN1530	TRP1538	ASP1586
10.	SS2(13)	GLN1530	TYR1537 TRP1538	GLU1534

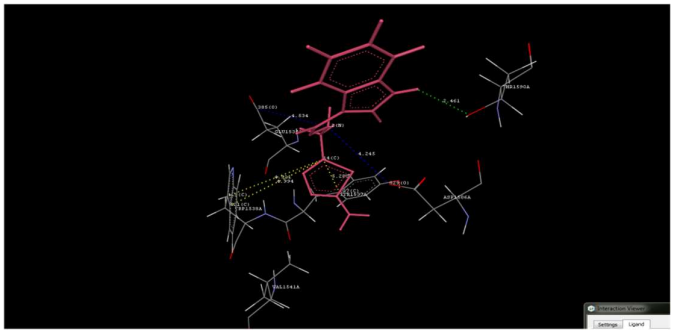

Figure 2: Docking Interaction of SS2(4)

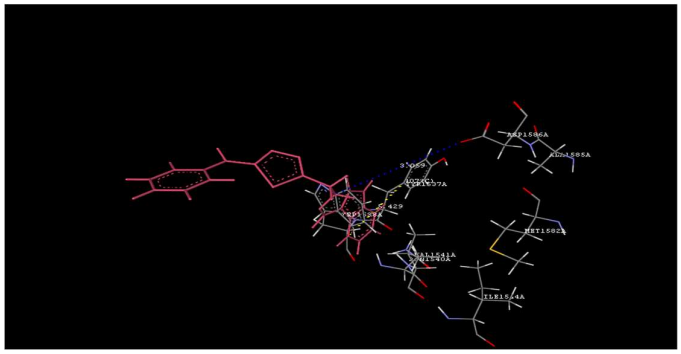

Figure 3: Docking Interaction of SS2(5)

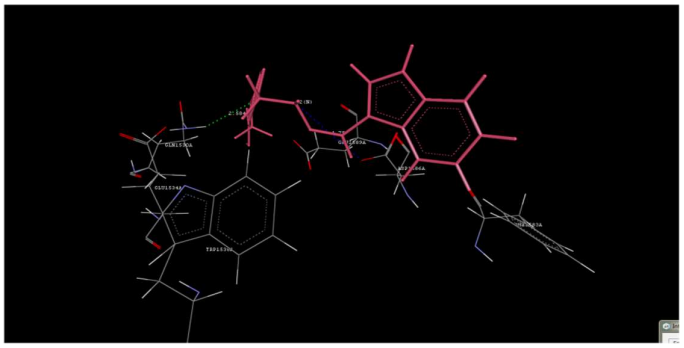

Figure 4: Docking Interaction of SS2(6)

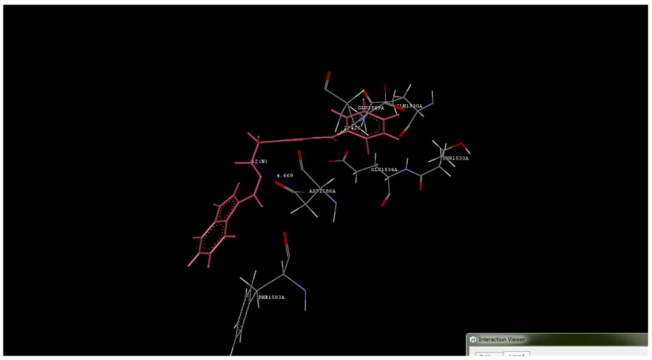

Figure 5: Docking Interaction of SS2(7)

Figure 6: Docking Interaction of SS2(8)

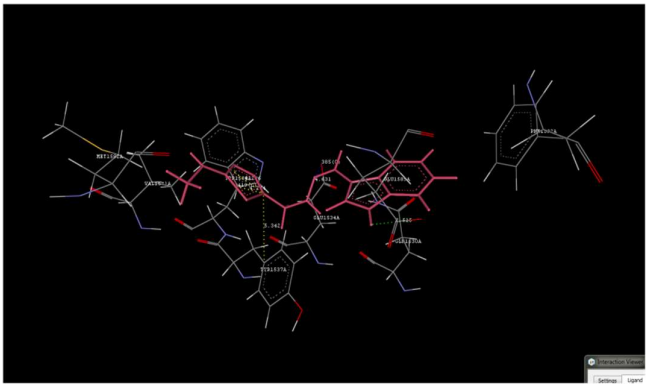

Figure 7: Docking Interaction of SS2(9)

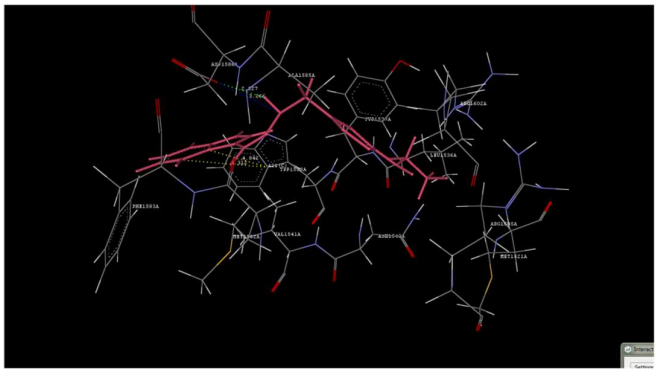

Figure 8: Docking Interaction of SS2(10)

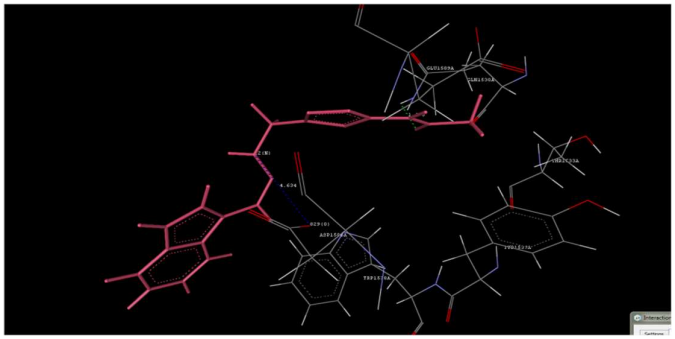

Figure 9: Docking Interaction of SS2(11)

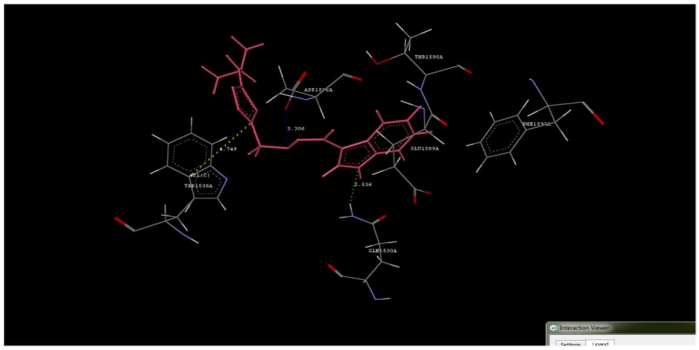

Figure 10: Docking Interaction of SS2(12)

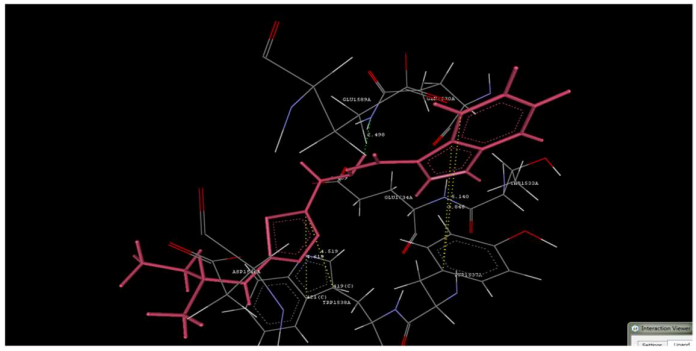

Figure 11: Docking Interaction of SS2(13)

Biological Activity of synthesised compounds:-

Behavioural study of synthesised compounds carried out by different animal model such as a Tail-flick method,Rota Rod co-ordination method Eddy's hot plate method for two synthesized compounds. It is found that, test sample compound SS2 (9) pointedly increase the tail flick time as compared to the standard group. Rats treated with SS2 (8) showed significant improvement in muscle grip strength and loco-motor activity on rota -rod as compared to the control and standard Group. Compound SS2 (8) also exhibited significant reduction in paw withdrawal latency as compared to standard group in STZ induced diabetic pain model.

Biochemical Estimation of synthesised comounds:-Biochemical estimation of synthesised compounds shows promising action such as The synthesized compounds SS2 (8) and SS2 (9) has decrease the LPO level indicate the significance of drug as anti-oxidant activity. The synthesized compound SS2 (8) significantly restore the catalase level as compared to control groups. Synthesized compounds SS2 (9) and SS2 (8) promisingly increase the GSH level as normal to standard group. This reflects the anti-oxidant property of synthesised compounds.

CONCLUSION:

Sodium Channel 1.7(Nav 1.7) is one of the promising biological targets for the development of the molecules against neuropathic pain. 10 different heterocyclic derivatives were prepared via the reaction of Indole 3 carboxy aldehyde. Molecules that are synthesized are characterized via spectral analysis. Virtual analysis of the synthesized derivatives was carried out to ascertain their potential against neuropathic pain. The synthesised molecule significantly increases the catalase, SOD, Glutathione peroxidase, glutathione level and lower the lipid peroxides level as compared to the disease state animal. It reflects the neuroprotective and anti-oxidant properties of synthesized compounds.

REFERENCES

[1] Arora P, Arora V, Lamba HS, & Wadhwa D, Importance of heterocyclic chemistry: a review, International Journal of Pharmaceutical Sciences and Research,2012, *3*(9), 2947.

[2] Manimaran T, Anand RM, Jishala MI, & Gopalasatheeskumar K,Review on substituted 1, 3, 4 thiadiazole compounds. IJPAR,2017, *6*(2), 222-231.

[3] Shamroukh AH, & Hegab MI, A review on synthesis, therapeutic, and computational studies of substituted 1, 3, 4 thiadiazole derivatives, Egyptian Journal of Chemistry,2020, *63*(11), 4387-4408.

[4] Verma G, Khan MF, Akhtar W, Alam MM, Akhter M, & Shaquiquzzaman M, A review exploring therapeutic worth of 1, 3, 4-oxadiazole tailored compounds, Mini Reviews in Medicinal Chemistry, 2020, *19*(6), 477-509.

[5] Siwach A, & Verma PK, Therapeutic potential of oxadiazole or furadiazole containing compounds. BMC chemistry,2020*, 14*, 1-40.

[6] Vaghani H, Patel S, & Patel S, 1, 3, 4-OXADIAZOLE AND ITS POTENCY: A REVIEW.

[7] Bhattacharya A, Wickenden AD., & Chaplan SR, Sodium channel blockers for the treatment of neuropathic pain. Neurotherapeutics,2009, *6*(4), 663-678.

[8] Hargus NJ, & Patel MK, Voltage-gated Na+ channels in neuropathic pain. Expert opinion on investigational drugs, 2007,*16*(5), 635-646.

[9] Matulenko MA, Scanio MJ, & Kort ME, Voltage-gated sodium channel blockers for the treatment of chronic pain, Current topics in medicinal chemistry,2009, *9*(4), 362-376.

[10] Ahuja S, Mukund S, Deng L, Khakh K, Chang E, Ho H, & Payandeh J,Structural basis of Nav1. 7 inhibition by an isoform-selective small-molecule antagonist. Science,2015, *350*(6267), aac5464.

[11] Sonvane S., Choudhari P., & Bhusnuare O, In silico analysis of polyphenols and flavonoids for design of human Nav1. 7 inhibitors, Journal of Biomolecular Structure and Dynamics,2021, *39*(12), 4472-4479.

[12] www.rcsb.org

[13] www.vlifesciences.com