"DESIGN AND DEVELOPMENT OF SOME NOVEL HETEROCYCLIC COMPOUNDS TARGETED FOR NAV1.7"

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Abstract:-

Pain is generally known as a disruptive sentiment which is results of forceful and damaging stimuli. Neuropathic pains (NP) are regularly associated with the chronic form which is result of the injury in the somatosensory nerves. 8-10% of the general population is found to be affected by neuropathic pain and it is normally associated with the sever clinical condition as more than 60 % of cases are associated with diabetic, cancer and other several injuries. To overcome of these need to synthesis novel heterocyclic compounds.1,3, 4-Oxadiazole ring has a crucial important in heterocyclic chemistry and have extensive utilization in screening for various biological activities. 1, 3, 4-Oxadiazole and Indole containing compounds have been gained considerable attention in pharmaceutical field due to their remarkable bioactivities. 6 different heterocyclic derivatives from Indole substituted Oxadiazole class were synthesized Molecules that are synthesized are characterized via by IR, Mass, C13 and H1 NMR spectral analysis. All the synthesized derivatives were found to be networking with Nav 1.7 which indicated further biological optimization of these compounds may lead to strong Nav 1.7inhibitors. Neuropathic pain activity of synthesized compound carried out by streptozocine (STZ) induced diabetic pain model.

Keywords: *Pain, Neuropathic pain, Oxadiazole, Docking, STZ.*

INTRODUCTION:-

Neuropathic pain (NP) is chronic and multifaceted neurological diseases which occur due to complete or partial destruction of nerve which leads to produce severe pain sensation. Tingling, numbness, shooting and burning sensation are the common symptoms of neuropathic pain. Neuropathic pain associated the 60% of human being who were suffering from chronic diseases such as diabetes, cancer and neurological disorders such as Alzheimer, epilepsy and Parkinson's diseases [1]. Variety of analgesic, aesthetic and anti-epileptic drug is used in treatment of neuropathic pain such as amitriptyline, Pregabalin, gabapentin, duloxetine, carbazepine, lidocaine, lamotrigine and opioids [2]. In short period Gabapentin and Pregabalin therapy is endorsed to determine the effectiveness of the drug in human being.62% of the person who takes the gabapentin may have at least one side effect and it response to the disease poorly. Literature suggests the need to develop a new dug with great efficiency with using appropriate neuropathic model[3]. In search of promising scaffold for treatment of NP, We thus review promising therapeutic targets that have emerged over the last 20 years, including Na^+, K^+, Ca^{2+}, hyperpolarization-initiated cyclic nucleotide–gated channels[4] .Many of the synthetic compounds give disappointing clinical trial results. Molecules which inhibit Na channels are effective as analgesic in the management of neuropathic pain. Different types of sodium channels like Nav1.3, Nav1.7, Nav1.8, Nav 1.9 plays significant role in the neuropathic pain management. Scientific studies revealed that NaV1.7 is required for initiation of pain signals [5].Therefore, a drug that selectively blocks the NaV1.7 channel has the potential to produce very strong analgesia for any kind of pain without the side effects associated with local anaesthetics, opioids, or NSAIDs [6 ,7,8]. A various kinds of heterocyclic compound are reach sources of scaffold for dealing with various chronic diseases. In this Indole Hetrocyclic compounds linked with Oxadiazole class of heterocyclic compounds has important therapeutically agents in pharmaceutical chemistry A literature suggests, the indole class of drug shows potent biological activity against anticancer, antioxidant, anti-HIV agent, neuropathic pain and neurological diseases such as Alzheimer, epilepsy and Parkinson's diseases and 1,3,4-Oxadiazole containing compounds have been gained considerable attention in pharmaceutical field due to their remarkable bioactivities [9]. These 3 compounds are effectively being utilized as antibacterial agents, anticancer, anti-Parkinson, anti-HIV, and anti-proliferative agents. In this research paper, we are reporting the synthesis of the 6 different heterocyclic molecules and their biological activity of active scaffold by streptozocine induced neuropathic pain model.

EXPERIMENTAL:-

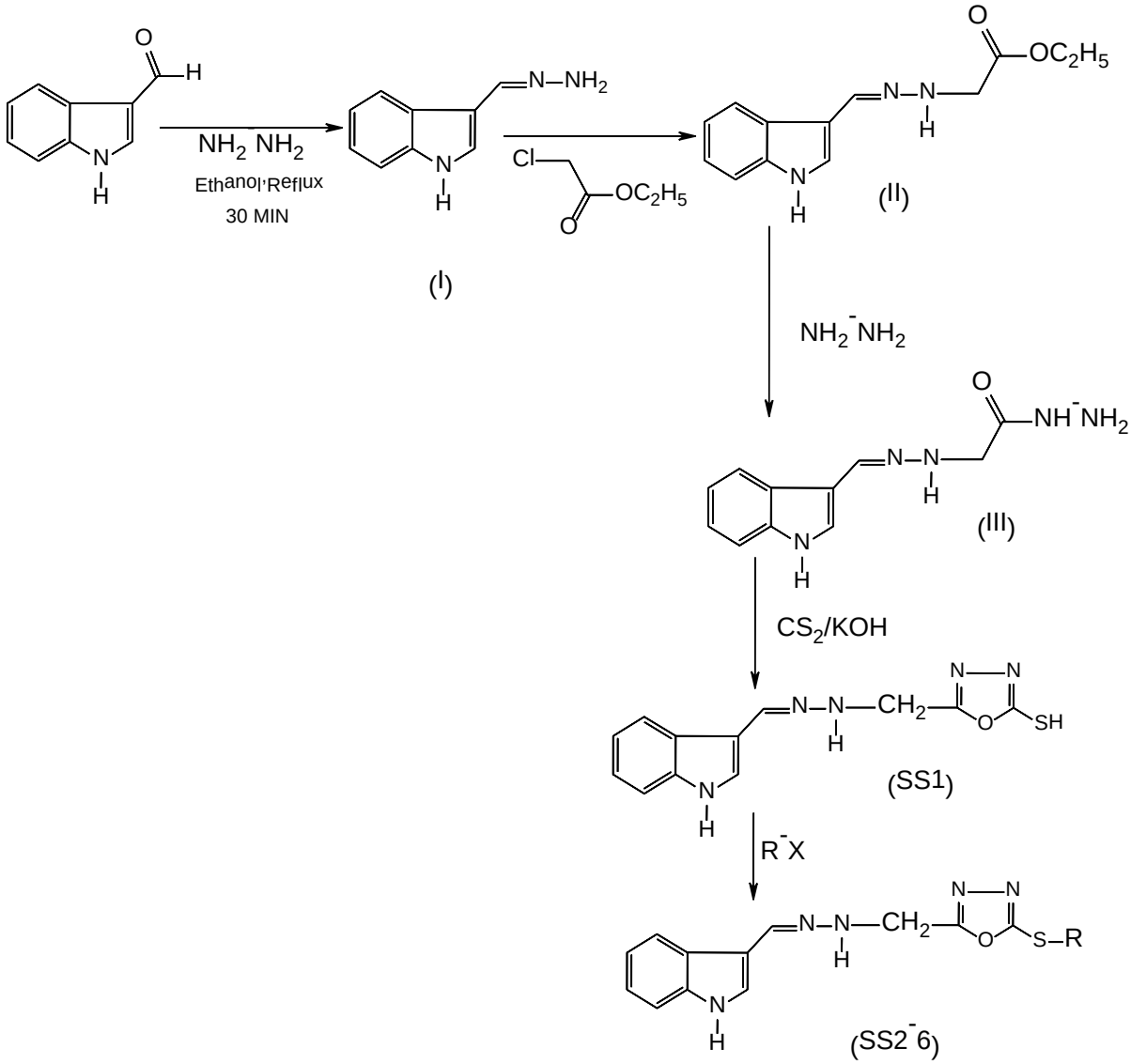

Figure 1. Scheme for synthesis of Heterocyclic compounds

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

Equimolar concentration of Indole-3-carboxaldehyde (0.01M) and hydrazine sulphate (0.01M) were refluxed in 25 ml ethanol for 6 hours in water bath. After completion of reaction the mixture poured in crushed ice .Filter the crude product and recrystallize the by using ethanol. Melting point -164^{0}C, R^f Value-0.58.Percentage yield:-62.93

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

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

Synthesis of 2-((2-(1H indol-3yl)methylene)hydrazinyl) acetohydrazide (III):-

A equimolar mixture of 1-(2-(1H-indol-3yl) methylene)hydrazinyl)butan-2-one (0.001M),hydrazine hydrate (0.01M) in 20ml of ethanol. Reflux the mixture for 10 hours. After completion of reaction mixture poured in crushed ice. Filter the crude product and recrystallize from ethanol. Melting point-240^0c.R^f value-0.66

Synthesis of 5-((2-((1H indol-3yl)methylene)hydrazinyl)methyl)-1,3,4-oxadiazole-2-thiol (SS1):-

A mixture of 2-((2-(1H indol-3yl)methylene)hydrazinyl)acetohydrazide (0.001M) and potassium hydroxide 0.8gm in absolute ethanol treated with 2ml of carbon disulphide. Reflux for 24 hours, after the completion of reaction mixture poured in crushed ice. Filter the crude product and recrystallize from ethanol. Melting point-172^0c.R^f value-0.63

Synthesis of 5-((2-((1H indol-3yl) methylene)hydrazinyl)methyl)-S-alkyl-1,3,4-oxadiazole-2-thiol (SS_{2-6}):-

A mixture of 5-((1H indol-3yl)methyl)-1,3,4-oxadiazole-2-thiol (0.001M),substituted alkyl halide (0.01M), 5ml sodium hydroxide (1%) in 25 ml of dioxane. Reflux the mixture for 30 hrs. Mixture poured in crushed ice. Filter the crude product and recrystallize 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) [10, 11]. Was downloaded from the free protein databank www.rcsb.org and utilized for docking analysis. Grip-based docking analysis was performed.

PHARMACOLOGICAL ACTIVITY:

The pharmacological screening of synthesized compounds carried out by streptozocine (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 to Group-IV SS3 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 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.

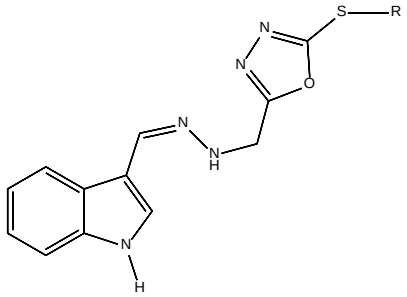

Table 1: Physicochemical data of the synthesized derivatives

Sr.	Compound	R-Group	Molecular	Molecular	Melting	
01	SS1	H	$C_{12}H_{11}N_5OS$	273	189	0.82
02	SS2	4-C_6H_5-COOH	$C_{19}H_{15}N_5O_3S$	393	193	0.70
03	SS3	-C_6H_5	$C_{18}H_{15}N_5OS$	349	169	0.68
04	SS4	CH_3-CH-C_2H_5	$C_{16}H_{19}N_5OS$	329	182	0.62
05	SS5	-CH_2-COOH	$C_{14}H_{13}N_5O_3S$	331	144	0.59
06	SS6	2-C_6H_5-Cl	$C_{18}H_{14}ClN_5OS$	383	210	0.76

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.

Spectral data of Synthetic compounds:-

Spectral data of Step- I

IR (KBr) cm^- :3200.07(NH stretching of NH_2), 3113.59(NH strhing), 1631.97 (C=N stretching);**^{1}H NMR :**([D,]DMSO): δ 7.19(d,2H -NH_2), 7.23-7.21-7.23 (d, 2H, Ar-H), 8.10-8.28(d, 2H, Ar-H), 8.08 (s, 1H,-CH), 8.92 (s, 1H,-CH), 9.93(s, 1H,NH),**13CNMR([D,]DMSO):** δ = 112-137 (Ar-C),138-154(=CH) ;**EIMS (M/z + 1) :** Molecular weight Correspond to 160**(m/z+1)** molecular ion peak.

v

Spectral data of step-II

IR (KBr) cm^{-} :3140(NH stretching), 1680.20(C=O Stretching); 1611 (C=N stretching);1575(aromatic C=C stretching);**^{1}H NMR :**([D,]DMSO): δ 1.31(s, 3H,-CH3),3.61(s, 2H,-CH2),4.23(s, 2H,-CH2),7.19(d,2H -NH_2), 7.22-8.08(Ar-H), 8.09 (s, 1H,-CH), 8.11 (s, 1H,-CH), 9.94(s, 1H,NH),12.15(s, 1H,NHIndole), **^{13}C 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

Spectral data of step-III

IR (KBr) cm- 3185(NH stretching), 1692(C=O Stretching); 1611 (C=N stretching); 1576(aromatic C=C stretching).**^{1}H NMR :(**[D,]DMSO): δ 2.60 (s, 2H,-NH2), 7.14-7.90 (Ar-H), 7.91 (s, 1H,-CH), 8.32 (s, 1H,-CH), 8..35(s, 1H,NH),8..89(s, 1H,NH),11.69(s, 1H,NHIndole) , **^{13}C NMR ([D,] DMSO):** δ 57.05(-CH2), 111.91-137.18 (Ar-C), 155.05(=CH) 184.92 (C=O), **EIMS (M/z) :** Molecular weight Correspond to 232.4 molecular ion peak

Spectral data of SS1

IR (KBr) cm^{-} :3400.35 (NH stretching), 1575.92(aromatic C=N stretching); 602.53(C-SH), **^{1}H NMR :**([D,]DMSO): 3.91(s,2H,-CH_2),7.14-8.32 (Ar-H), 8.35 (s, 1H,-CH), 8..90(s, 1H, SH),11.69(s, 1H,NHIndole) , **^{13}C NMR ([D,] DMSO):** δ = 49.05 (-CH_2), 111.91-137.19 (Ar-C), 155.05(=CH), **EIMS (M/z) :** Molecular weight Correspond to 273.07 molecular ion peaks.

Spectral data of SS2

IR (KBr) cm^{-} 3400 (NH stretching), 3100 (-OH stretching) 2919 (C-C aliphatic stretching), 1720(C=O Stretching) 1590(aromatic C=C); 685(C-S stretching);**^{1}H NMR :**([D,]DMSO): δ1.79(s, 2H-CH2), 7.19-8.32 (q, Ar-H), 7.91 (s, 1H,-NH),8.35(s,-CH), 8.90 (s, 1H,NHIndole) 12,03 (s, 1H,-OH);**^{13}C NMR ([D,] DMSO):** δ 60.64(-CH_2), 112.03-139.18 (Ar-C), 155.05(=CH), 168.67(C=O), **EIMS (M/z):** Molecular weight Correspond to 394 molecular ion peak.

Spectral Data of SS3

IR (KBr) cm^{-} 3100 (NH stretching), 2916 (C-C aliphatic stretching), 1620(aromatic C=C); 568(C-S stretching), **^{1}H NMR :**([D,]DMSO): δ1.10-1.12(s, 2H-CH2), 7.19-7.50 (q, Ar-H), 7.99 (s, 1H,-NH), 11.82 (s, 1H,NHIndole), **EIMS (M/z) :** Molecular weight Correspond to 327.4 molecular ion peak.

Spectral Data of SS4

Compound Code SS2 (10):- IR (KBr) cm^{-} 3180 (NH stretching), 2916 (C-C aliphatic stretching), 1613(aromatic C=C); 640(C-S stretching),

Spectral Data of SS5

Compound Code SS2 (11): IR (KBr) cm^{-} 3140 (NH stretching), 3000(OH stretching) 2916 (C-C aliphatic stretching), 1695(C=O stretching), 1590(aromatic C=C); 647(C-S stretching).

Spectral Data of SS6

Compound Code SS2 (12): IR (KBr) cm^{-} 3183 (NH stretching), 2916 (C-C aliphatic stretching), 1611(aromatic C=C); 706(Ar-Cl), 646(C-S stretching)

MOLECULAR DOCKING:-

Molecular docking was accomplished to evaluate the binding affinity of the designed scaffolds with sodium Channel NAV 1.7 .Structure of the Sodium Channel NAV 1.7 was taken from the free protein databank www.rcsb.org used for docking study. Grip based docking analysis was performed. Derivative SS2 (3) was establish hydrogen bond interaction with MET1582 and aromatic interactions with TYR1537, TRP1538. Derivative SS3 was created hydrogen bond interaction with GLN1530 and aromatic interactions with TYR1537, TRP1538. Derivative SS2 was established hydrogen bond interaction with ASP1586 and aromatic interactions with TYR1537, TRP1538. Derivative SS4 was created hydrogen bond interaction with GLN1530 and aromatic interactions with TYR1537, TRP1538. SS5 interacted via hydrogen bond interaction with GLN1530, SS6 Showed hydrogen bond interaction with TRP1538 and two aromatic interactions with TYR1537 ASP1586[12,13]. Docking interaction of designed ligands shown in table.

Table 2: Docking Interactions of synthesized Molecules

Sr. No	Molecule No	Interaction		
		H bond	Aromatic	Charge
1	SS1	MET1582	TYR1537 TRP1538	-
2	SS2	ASP1586	TYR1537 TRP1538	-
3	SS3	GLN1530	TYR1537 TRP1538	-
4	SS4	GLN1530	TYR1537 TRP1538	-
5	SS5	GLN1530	-	-
6	SS6	TRP1538	TYR1537 ASP1586	-

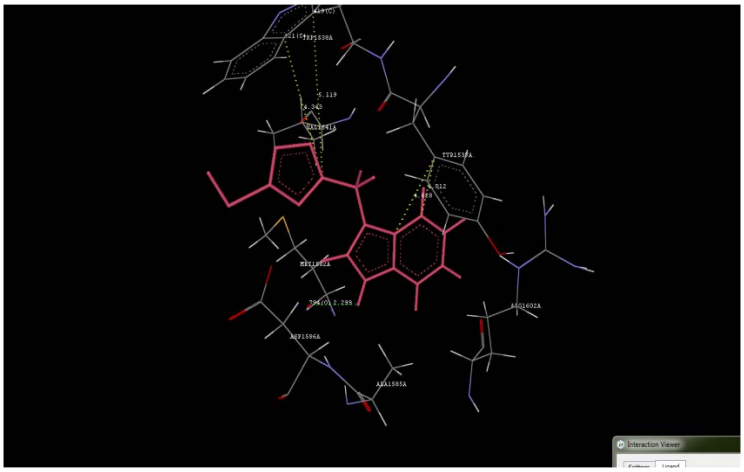

Figure 2. Docking Interaction of SS1

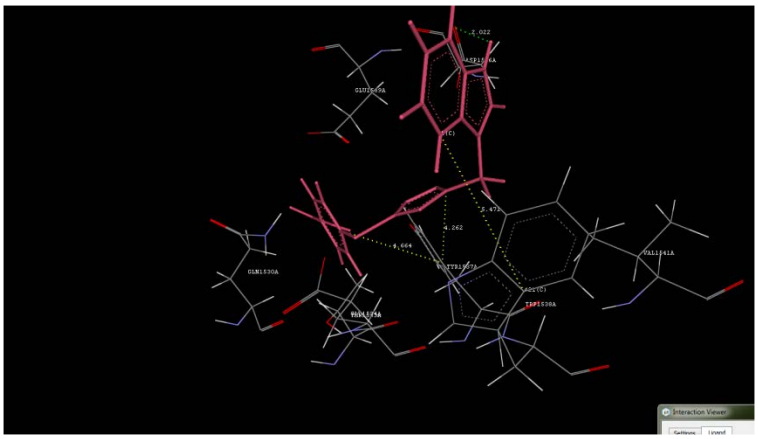

Figure 3. Docking Interaction of SS2

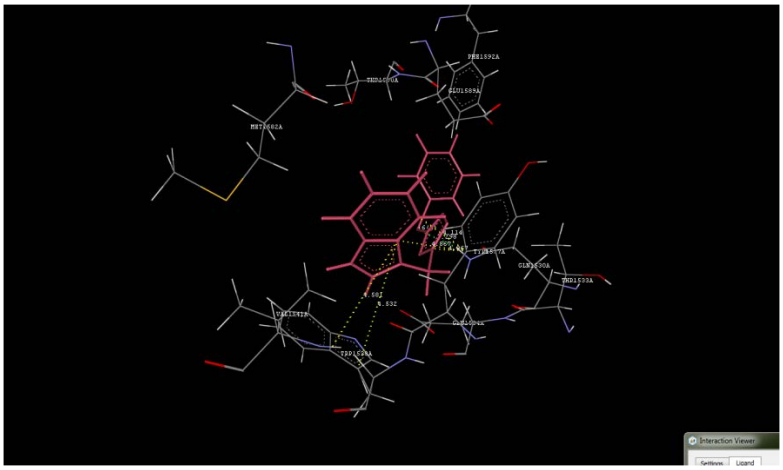

Figure 4. Docking Interaction of SS3

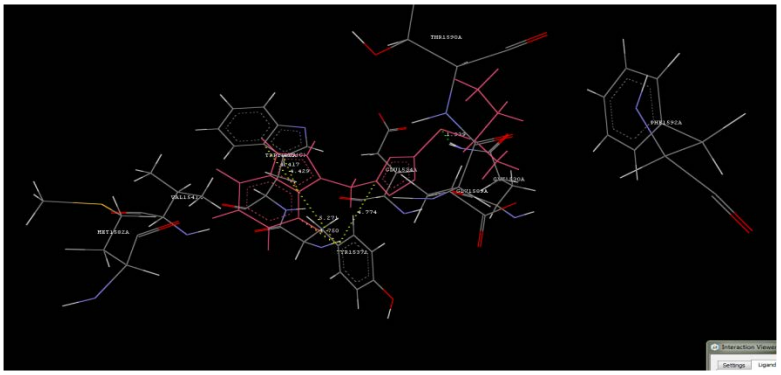

Figure 5. Docking Interaction of SS4

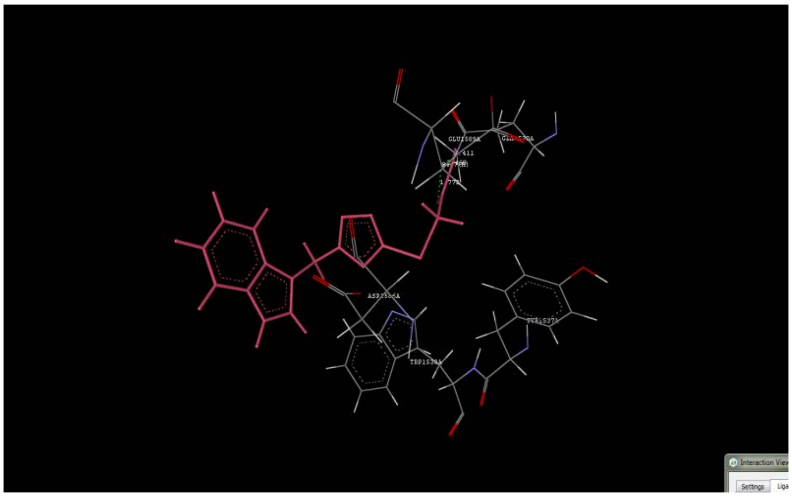

Figure 6. Docking Interaction of SS5

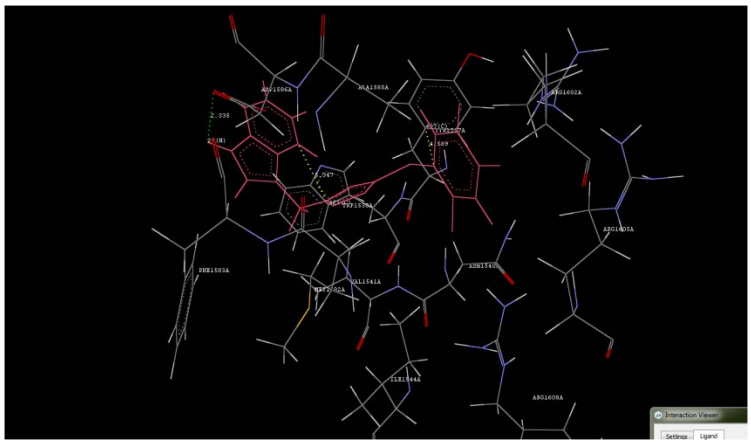

Figure 7. Docking Interaction of SS6

Biological Activity of synthesised compounds:-

Behavioural study of synthesised compounds carried out by different animal model such as a Tail-flick method, Eddy's hot plate method for two synthesized compounds. Whereas compound SS2 (2) and SS2 (3) is shows similar effect with control group animals in hot plate allodynia test. Compound SS2 (3) shows good result as compare to standard pregabline group in hot plate hyperalgesia test. In tail flick method test sample compound SS2 (2) pointedly increase the tail flick time as compared to the standard group.

Biochemical Estimation of synthesised cofounds:-Biochemical estimation of synthesised compounds shows promising action such as the synthesized compounds SS2 (2) [0.724 ±0.016**] and SS2 (3) [0.737 ±0.009*] has decrease the LPO level indicate the significance of drug as anti-oxidant activity as compared to control group [0.820 ±0.021]. The synthesized compound SS2 (2) [2.36 ±0.11**] and SS2 (3)[2.30 ±0.17*] significantly restore the catalase level as compared to control groups [2.65 ±0.10]. SOD and CAT contribute jointly acts in defence of reactive oxygen species. The synthesized compound SS2 (3) [2.84 ±0.09*] restored the SOD level to near to normal level. The present study reveals that synthesized compound SS2 (3) [0.80±0.02***] and SS2 (2) [0.70±0.01***] has promisingly increase the glutathione peroxidase level in sciatic nerve of disease rat [0.35±0.02]. It reflects the neuroprotective and anti-oxidant properties of synthesized compounds. Synthesized compounds SS2 (3) [2.09 ±0.08**] 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 favourable biological targets for the development of the molecules against neuropathic pain. 06 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.

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