



Research Article

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Anticonvulsant, analgesic and anti-inflammatory activities of some novel pyrrole and 1,4-dihydropyridine derivatives

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ABSTRACT

In this work we reported the synthesis some pyrrole and 1,4-dihydropyridine derivatives and evaluation of pharmacological activity. Synthesized compounds were confirmed by IR, ¹H NMR, ¹³C NMR, Mass spectral and elemental analysis. Synthesized compounds were screened for in vivo Pharmacological activity such as Anticonvulsant, Analgesic and Anti-inflammatory activities. Among the pharmacological screening the compound 4 showed that better activity against anti-inflammatory when compared with the standard drugs (Diclofenac sodium). Molecular docking studies of synthesized compounds and diclofenac were designed by binding ability against COX-1, COX-2 enzymes. The molecular docking results emphasized the in vivo data and all docked molecules were found to display very high binding constant compared to standard drug. Structure activity relationships (SAR) were discussed in this paper.

Keywords: Anticonvulsant activity, Analgesic activity, Anti-inflammatory activity, 1,4-dihydropyridine derivatives, Pyrrole derivatives, Structure activity relationship.

INTRODUCTION

In recent years, the high therapeutic properties of thiosemicarbazone and their heterocyclic related drugs have been attracting the attention the medicinal chemists. Pyrrole and 1,4-dihydropyridine derivatives represent a class of compounds of great importance in heterocyclic compound. Pyrrole derivatives are of interest because of their potential biological activity such as anticoagulant [1], antimicrobial [2,3] COX-1/COX-2 inhibitors [4], human tumor models [5], anticonvulsant [6], antiviral [7] and anticonvulsant activities [8]. 1,4-dihydropyridine derivatives also have important of pharmacological activity such as antihypertensive[9], anti-ischemic drugs [10], calcium channel modulators [11], anticonvulsant [12], anticancer[13], anticoagulant [14] and antimicrobial activities [15]. Based on above observation, Current investigation of this work synthesis of new pyrrole and 1,4-dihydropyridine derivatives and evaluated for pharmacological activity.

EXPERIMENTAL SECTION

2.1. General

Melting points were recorded in open capillary tubes and were uncorrected. The IR spectra (KBr) were recorded on a Shimadzu 8201pc (4000-400 cm⁻¹). The ¹H NMR was recorded on a Bruker DRX-400 MHz . The Elemental

analysis (C, H, N and S) were recorded using an Elementer analyzer model (Varian EL III). The purity of the compounds was checked by thin layer chromatography (TLC) with silica gel plates.

3.2. Chemistry

2.2. Synthesis of 2,2'-[(3,5-dimethyl-1H-pyrrole-2,4-diyl)dicarbonyl] dihydrazine carbothioamide (2).

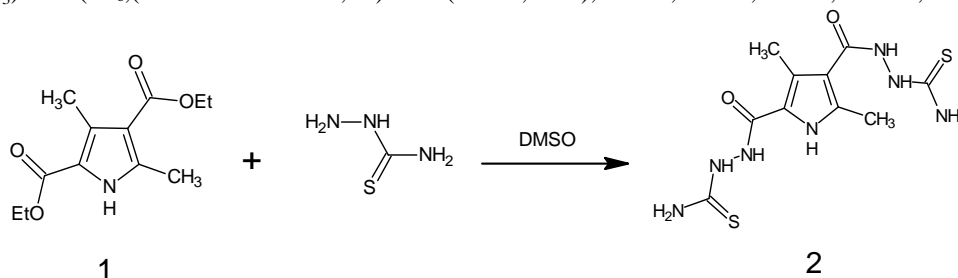
A mixture of the compound (1) (0.1 mol) and thiosemicarbazide (0.2 mol) in ethanol and add few drop of DMSO, the reaction mixture was reflux for 7 h. The reaction mixture was poured in to crushed-ice. The predicated was collected by filtration and recrystallised by absolute ethanol.

IR (KBr, cm^{-1}) ν := 3354(NH), 3241(NH₂), 1747(C=O), 1265(C=S), 1084(N-C-N). ¹H-NMR (DMSO-d₆): δ 11.88 (s, 1H, NH of pyrrole ring), 10.41 (d, 2H, 2xCONH), 9.51 (s, 2H, NH₂), 2.32 (s, 2H, CH₃), 2.10 (s, 3H, CH₃), 1.71 (d, 2H, 2 x NHCS). ¹³C-NMR(DMSO-d₆): δ 185.69 (2x CS NH₂), 165.76, 161.71 (2,4-CONH), 144.15, 141.16, 128.46, 115.50, 18.22, 11.74. MS (m/z, (relative abundance, %): 329.22 [M⁺, 31%], 270.42(70%), 212.46 (100%), 151.16 (34.1%), 96.07(13%).

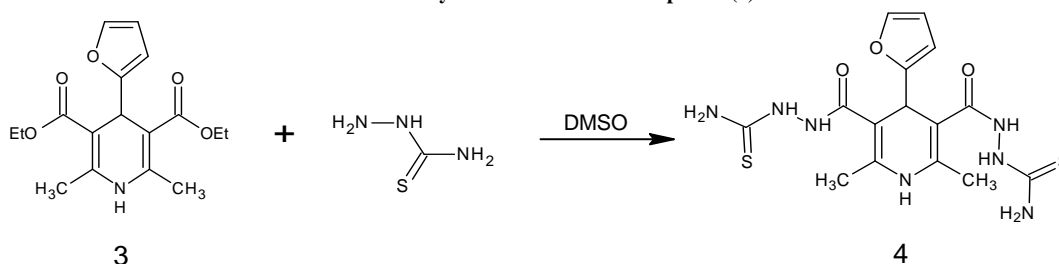
2.3. Synthesis of 2,2'-[[4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-diyl] dicarbonyl]dihydrazinecarbothioamide (4)

A mixture of the compound (3) (0.1 mol) and thiosemicarbazide (0.2 mol) in ethanol and add few drop of DMSO the reaction mixture was reflux for 7 h. The reaction mixture was poured in to crushed-ice. The predicated was collected by filtration and recrystallised by absolute ethanol.

IR (KBr, cm^{-1}): 3372 (N-H), 3218 (NH₂), 3200 (-NH-CO-), 3021 (Ar-H), 1260 (C=S), 1091 (N-C-N), 828 (Ar-H). ¹H-NMR (300 MHz, CDCl₃, δ / ppm): 9.62 (s, 2H, NH₂), 8.43 (s, 1H, NH of pyridine ring), 8.09 (d, 2H, 2 x CONH), 7.51 (d, 1H, 5'-H-furyl), 6.24 (d, 1H, 4'-H-furyl), 6.24 (d, 1H, 3-H-furyl); 5.17 (s, 2H, C4-H), 2.28 (s, 6H, 2x CH₃), 2.02 (d, 1H, NHCS). ¹³C -NMR (300 MHz, CDCl₃, δ / ppm): 111.8, 108.3, 143.2, 152.8 (C4 in furyl ring), 105.3 (C3,5 in pyridine ring), 166.2 (C=O), 182.1 (C=S), 148.9 (C2,6 in pyridine ring), 35.3(C4 in pyridine ring), 18.2 (2 x CH₃). MS (m/z, (relative abundance, %): 410(M⁺+1, 30.2), 291.30, 161.27, 175.22, 147.12, 81.11.



Scheme 1: Synthetic route of the compound (2)



Scheme 2: Synthetic route of the compound (4)

2.4. PHARMACOLOGY ACTIVITIES

2.5. Anticonvulsant activity

Synthesized compounds (1-4) were screened for their anticonvulsant activity against the pentylenetetrazole induced convulsions in Swiss albino-rats. Rats are weighing 150g divided in to 4 groups containing 5 animals in each group, normal saline and treated as normal control, standard drug phenytoin (10mg/kg). The test compounds are dissolved in DMSO and i.p at a dose of 10mg/kg. The test was evaluated by maximal electrode induced convulsion method [19, 20].

2.6. Analgesic activity

Synthesized compounds (1-4) were screened for their analgesic activity, albino mice weighing 25-30g were used in the present study. The laboratory temperature was minted at a 12h light dark schedule. Before experimentation mice were allowed 1 week of adaptation. The animals were divided in to four groups. Each groups included 5 animals. All compounds were dissolved in DMSO and given to the animals at 10mg/kg dose in approximately 0.1mL

volume, control animals were received 0.1mL DMSO p.o. Tail flick test to radiant heat [21,22]. Tail flick test to radiant heat Analgesia was measured by the tail flick test to radiant heat. This test was done with beam of high intensity light focused on the dorsal surface of the tail. The response latency between the one set of radiant heat simultaneous. The tail flick response in second was taken end point of equipment. Digital analgesio meter (tail flick method 1Nco) and convert of 5.4 amp is applied. The light intensity was set to provide a pre drug response time of 2-4h. A cut-off 10-15 seconds was used in order to prevent damage to the tail.

2.7. Anti-inflammatory activity

Synthesized compounds (1-4) were screened for their anti-inflammatory activity, by literature method [23]. Albino rats of both sexes weighing 150g were divided in to 4 groups, each group consists of 5 animals. Inflammation was induced by intra planter injection of Histamine (0.1mL of 1% Histamine for induction of pawedema). The rats are challenged by s.c injection of 0.1mL of 1% solution of histamine in to the sub-plantar side of the left hind paw. Percentage of anti-inflammatory activity = $(Vc - Vt / Vc) \times 100$. 1h after the administration of the test compounds (10mg / kg; p.o), one group was kept as control, received only 0.5% carboxy methyl cellulose solution. The right hind paw volume was measured before and after 3h of carrageen treatment by means of plethysmometer.

2.8. Molecular docking

Synthesized compounds were designed by chemoffice2004 software and docking simulation was carried out to all the designed compounds against 1Q4G (COX-1 enzyme), 6COX (COX-2 enzyme) with the help of arguslab program and the docking scores of each compound was analyzed. Compounds showed very good interaction energies (Table 5) even better than that of diclofenac acid.

RESULTS AND DISCUSSION

3.1. Chemistry

Diethyl 3,5-dimethyl-1*H*-pyrrole-2,4-dicarboxylate **1** was prepared by Fischer condensation method [16] and compound (diethyl 4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) **3** was synthesized from Hantzsch method [17]. The 2,2'-[(3,5-dimethyl-1*H*-pyrrole-2,4-diy)] dicarbonyl dihydrazine carbothio amide **2** and 2,2'-{[4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-diy] dicarbonyl}dihydrazinecarbothioamide **4** were prepared by condensation method [18]. The physicochemical characteristics of the compounds are presented in (Table 1). The IR spectra of compound **2** shows an absorption band at 3354, 1747, 1265, 3241 and 1084cm⁻¹ corresponding to NH, C=O, C=S, NH₂ and C-N-C groups respectively. The ¹H NMR spectra of compound **2** shows that signals at δ 9.51, 11.88, 10.41 and 1.71 corresponding to NH₂, NH, 2,4-CONH and 2,4-NHCS protons respectively. ¹³C NMR spectra of compound **2** shows that peaks at δ 165.76, 161.71 and 185.69 corresponding to C4,2-CONH and 2,4-CSNH₂ carbons respectively. The mass spectrum of the compound **2** shows that molecular ion peak at m/z 329.22 corresponding to molecular mass of compound **2**. The IR spectra of compounds **4** shows an absorption band obtained at 3372, 3200 and 1260 cm⁻¹ corresponding to the NH, NH-CO- and C=S group respectively. ¹H NMR spectra of compound **4** shows that singlet at δ 8.43, 5.17, 8.09, 2.02 and 9.62 ppm corresponding to the NH, C4-H, CONH, NHCS and NH₂ protons respectively. ¹³C NMR spectra of compound **4** shows that peaks at δ 166.2, 182.1, 35.3 and 18.2 ppm, corresponding to CO-NH, C=S and CH₃ carbons respectively.

Table 1: Physicochemical properties of compounds (1-4)

| Com.No | Mp (°C) | M.w | Yield (%) | M.F. | Elemental Analysis Calculated (Found) % | | | |
|----------|---------|--------|-----------|--|---|-------------|---------------|---------------|
| | | | | | C | H | N | S |
| 1 | 127 | 239.27 | 71 | C ₁₂ H ₁₇ NO ₄ | 60.24 (60.28) | 7.16 (7.13) | 5.85 (5.81) | - |
| 2 | 95 | 329.40 | 85 | C ₁₀ H ₁₅ N ₇ O ₂ S ₂ | 36.46 (36.40) | 4.59(4.51) | 29.77 (29.72) | 19.47 (19.41) |
| 3 | 158 | 319.35 | 91 | C ₁₇ H ₂₁ NO ₅ | 63.94 (63.91) | 6.63 (6.69) | 4.39 (4.41) | - |
| 4 | 214 | 409.48 | 88 | C ₁₅ H ₁₉ N ₇ O ₃ S ₂ | 44.00 (44.06) | 4.68 (4.74) | 23.94 (23.98) | 15.66 (15.71) |

Table 2: Anticonvulsant activity of compounds (1-4)

| Comp. No. | Duration of convulsion (s) | Percentage of activity(%), Dose (10 mg/ kg) |
|-----------|----------------------------|---|
| Control | 63.6 | - |
| 1 | 50.2* | 21 |
| 2 | 21.8* | 66 |
| 3 | 49.6* | 28 |
| 4 | 10.0* | 84 |
| Standard | 1.2* | 98 |

Dose (10 mg/ kg)

*P > 0.001 Vs Control

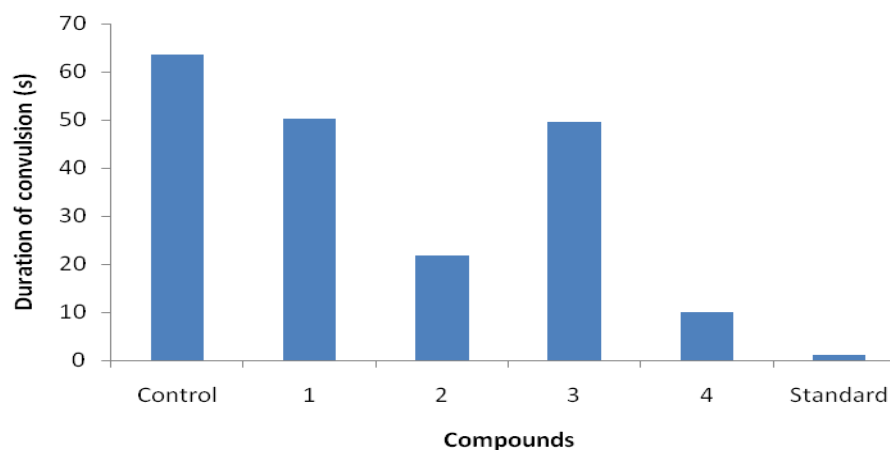
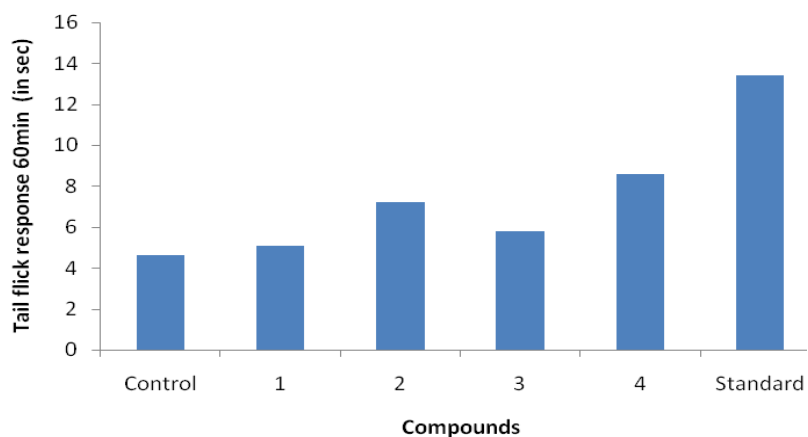
Phenytoin as a standard

Table 3 : Analgesic activity of the compounds (1-4) by tail flick response method

| Comp. No. | Tail flick response | |
|-----------------|---------------------|------------------------------------|
| | 60min (in sec) | % of activity, Dose (10 mg/ kg) |
| Control | 4.6 | - |
| 1 | 5.1 | 34 |
| 2 | 7.2 | 48 |
| 3 | 5.8 | 38.6 |
| 4 | 8.6 | 57.3 |
| Standard | 13.4 | 89.3 |

* $p > 0.001$ vs controlThe maximum tail flick response is 15sec, it is considered as 100%
Pentazocine as a standard**Table 4: Anti-inflammatory activity of compounds (1-4)**

| Comp. No | Increase in paw volume (3hr – 0hr) | Percentage (%) of |
|-----------------|---------------------------------------|-------------------------------|
| | | Activity, Dose (10 mg/ kg) |
| Control | 0.56 | - |
| 1 | 0.27* | 51.7 |
| 2 | 0.18* | 67.8 |
| 3 | 0.30* | 46.4 |
| 4 | 0.16* | 71.4 |
| Standard | 0.20* | 64.2 |

Significance levels * $P > 0.001$ as compared with the respective control.
Diclofenac sodium was used as a standard**Figure 2: Anticonvulsant activity of compounds (1-4)****Figure 3: Analgesic activity of compounds (1-4)**

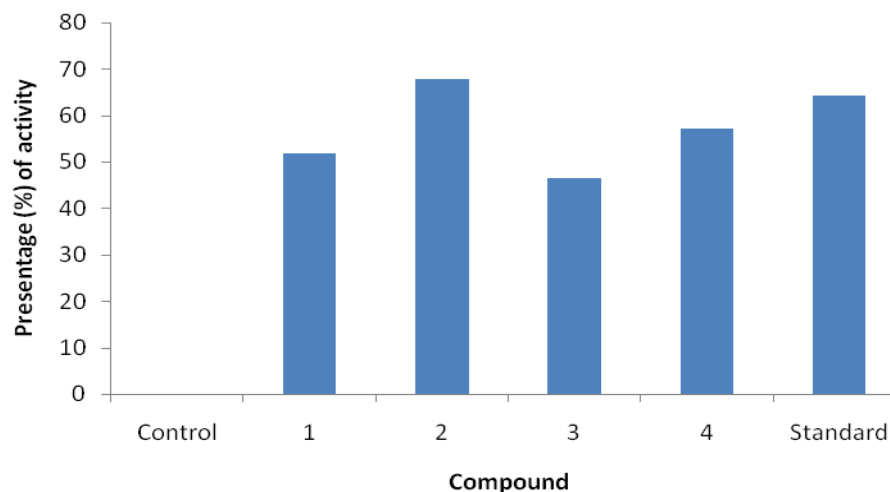


Figure 4: Anti-inflammatory activity of compounds (1-4)

Table 5: Docking scores (kcal/mol) of synthesized compounds (1-4)

| Ligand | Binding Energy (COX-1), Kcal/mol | Elapsed time (Sec) | Binding Energy (COX-2), Kcal/mol | Elapsed time (Sec) |
|----------|----------------------------------|--------------------|----------------------------------|--------------------|
| 1 | -8.263 | 5 | -7.211 | 6 |
| 2 | -7.377 | 5 | -7.496 | 5 |
| 3 | -9.027 | 7 | -7.630 | 8 |
| 4 | -8.327 | 6 | -8.360 | 8 |
| Standard | -8.380 | 2 | -3.691 | 4 |

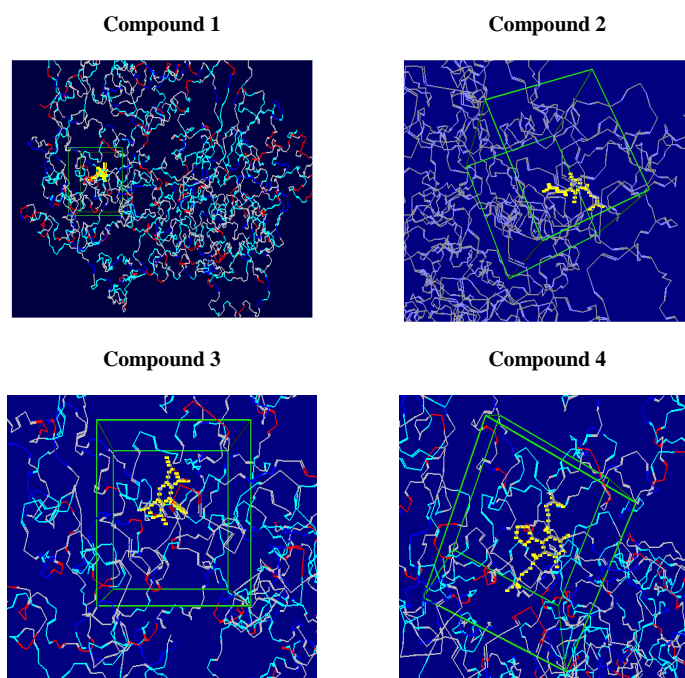


Figure 5: Binding modes of designed ligands with COM-1(1Q4G)enzymes

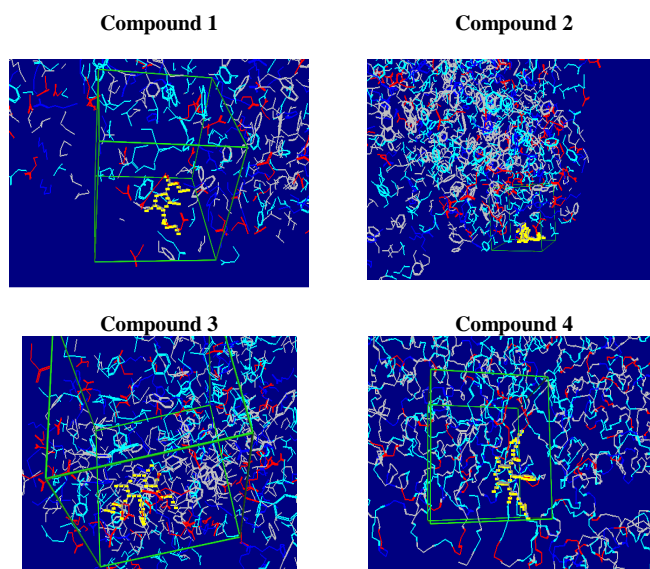


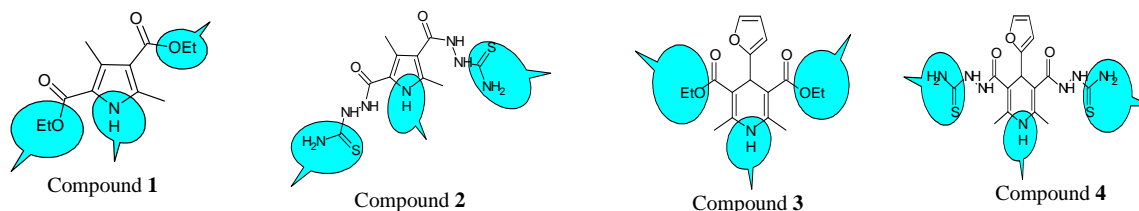
Figure 6: Binding modes of designed ligands with COM-2 (6COX)enzymes

3.2. Anticonvulsant activity

The compounds **1-4** were screened for anticonvulsant activity the compounds were tested at concentration 10mg/kg. Phenytoin was used as standard. All compounds were less active compared with standard whereas compound **4** has highly active compared with other compounds **1-3**. (Fig. 2) shows that activity variation of compounds **1-4** anticonvulsant activity data are presented in (Table 2).

3.3. Analgesic activity

The compounds **1-4** were screened for analgesic activity; the compounds were used at concentration 10mg/kg. Pentagocine was used as standard. All compounds were less active compared with standard whereas compound **4** has highly active when compared with compounds **1-3**. (Figure 3) shows that activity variation of compounds **1-4**, analgesic activity data are presented in (Table 3).



3.4 Anti-inflammatory activity

The compounds **1-4** were screened for anti-inflammatory activity the compounds were compared with standard drug diclofenac sodium at oral dose. Rat albino was used oral dose of the test compound at concentration 10mg/kg, the percentage of the activity was measured at 3h. Compound **4** has highly activity compared with standard. (Fig. 4) shows that activity variation of compounds **1-4**, anti-inflammatory activity data are presented in (Table 4).

The binding ability of this newly designed ligands with COX-1, and COX-2 enzymes were determined with the help of molecular docking studies using arguslab program. (Fig.1) Structure activity relationship. The binding scores of designed ligands **1-4** with COX-1 enzymes ranging from -7.377 to -9.027 Kcal/mol and COX-2, enzymes ranging from -7.211 to -8.360 respectively (Table 5) and the binding mode of ligands with COX-1, COX-2, and enzymes was showed in (Fig. 5, 6). These data clearly indicates their potency as cyclooxygenase inhibitors. Almost all the designed ligands showed good interaction energy than the diclofenac which showed the following interaction energies -9.027 Kcal/mol and -8.360 Kcal/mol with COX-1, COX-2 enzymes respectively.

3.5. Structural activity relationship

From the results of Pharmacological activities in pyrrole and 1,4-dihydropyridine derivatives, the following structure activity relationships can be derived. Compound **1** containing pyrrole with -OEt group shows that low activity against anticonvulsant, analgesic, and anti-inflammatory activities where as compound **2** containing pyrrole

with $-CSNH_2$ group shows that highly activity compared with compound **1** against anticonvulsant, analgesic, and anti-inflammatory. Compound **3** containing pyridine with $-OEt$ group shows that low activity against anticonvulsant, analgesic, and anti-inflammatory activities where as compound **4** containing pyridine with $-CSNH_2$ group shows that highly activity compared with compound (**3**) against anticonvulsions, analgesic and anti-inflammatory. In conclusion anticonvulsant, of pyrrole compounds **1,2** shows that less active against analgesic activity compared with 1,4-dihydropyridine compounds **3,4** whereas pyrrole compounds **2, 4** shows that highly active against anti-inflammatory activity compared with 1,4-dihydropyridine compound **1,3**. (Figure 1) shows that structure activity relation of compounds **1-4**.

CONCLUSION

In conclusion Synthesis of thiosemicarbazone and their Pyrrole and 1,4-dihydropyridine derivatives demonstrate moderate and significant pharmacological activity. Among them compound **4** displayed highest activity in anti-inflammatory compared with standard Diclofenac sodium at concentration 10mg/kg. The results reported here provide a foundation of further development of Pyrrole anti-inflammatory drugs.

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