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Synthesis, characterization of quinoxaline-2, 3-dione tethered 2-amino-5-substituted-1, 3, 4-thiadiazole derivatives as anti-inflammatory agents

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Abstract
Aim: The aim of the present study is to synthesis and screening the anti-inflammatory effect quinoxaline-2-3-dione tethered 2-amino-5-substituted-1,3,4-thiadiazole derivatives.
Method: Thus various 2-amino-5-substituted-1,3,4-Thiadiazole were added to a mixture of formaldehyde and 75% ethanol along with quinoxaline-2,3-dione then irradiated for 4 minutes at 250 watt. All the title compounds were tested for their anti-inflammatory activity of the synthesized compounds was evaluated by Carrageenan induced paw edema method in rats. The activity was studied at 200 and 400 mg/kg b.w., p.o. and their effects were measured at 30, 60, 120 and 180 min.
Result: Anti-inflammatory activity of synthesized compounds was tested by Carrangeenan induced paw edema method in rats standard drug used for the study was Diclofenac sodium. The methoxy, chloro and bromo substitution in three derivatives showed significant anti-inflammatory activity when compared to standard. The hydroxy, nitro groups showed mild activity when compared to standard. Conclusion: A novel series of quinoxaline 2,3- Dione derivatives has anti-inflammatory activity so supplementary exploration has been needed to establish other pharmacological performance of these derivatives and in future it would be a most promising and leading derivatives for several ailments.

Keywords: quinoxaline2, 3-dione, thiazolidines, anti-inflammatory activity, schiff bases reactions

Introduction

The aim of the present study is to synthesis and screening the anti-inflammatory effect of novel series of quinoxaline-2-3-dione tethered 2-amino-5-substituted-1,3,4-thiadiazole derivatives.

Among the various classes of nitrogen-containing heterocyclic compounds, quinoxalines display a broad spectrum of biological activities [1]. Many quinoxaline derivatives have been reported to possess diverse pharmacological activities such as antimicrobial [2, 6]. anticancer [7]. anti-inflammatory [8] antidiabetic [9]. Antiepileptic [10]. and antidepressant properties [11]. Quinoxaline is a class of antibiotics is disclosing potent antibacterial, anticancer and antiviral activities. The latter is a quinoxaline-containing drug targeting DNA as bifunctional intercalating agents [12]. Quinoxaline antibiotics show diverse inhibitory activities against enzymes, namely DNA polymerase, RNA polymerase, reverse transcriptase and topoisomerase [13]. A literature survey indicates that a quinoxaline derivative possesses different pharmacological and biological activities, which of most potent activity is antimicrobial anti-fungal, anti-inflammatory and analgesic activity. We thought to synthesize novel substituted quinoxaline moiety. Quinoxaline and its mode of action that prevent DNA-directed RNA synthesis by virtue of binding to cpG site on DNA. 4-Thiazolidinone derivatives are known to possess antimicrobial [14, 16] and anti-inflammatory [17, 19] properties. 4-thiazolidinone has been reported as novel inhibitors of the bacterial enzyme, which is precursor acting during the biosynthesis of peptidoglycan.

Methods and materials

General procedure for synthesis: (Under Microwave Irradiation)

Step 1 [Synthesis of Quinoxaline-2,3-dione]

A powdered mixture of oxalic acid dihydrate (0.01 mole, 1.26 g) and o-phenylene diamine (0.01 mole, 1.0814 g) was put in an open beaker, and 1 ml of water added and mixed thoroughly. The mixture was irradiated in a catalyst microwave system at an emitted power of 400 W for 3 min **[20]**. 100 ml of water was added, followed by further irradiation for 1 min to give a clear solution and then left to stand at room temperature. The product obtained was filtered, washed with water, and recrystallized with suitable solvent.

Step 2 [Synthesis of 2-amino-5-substituted-1,3,4-thiadiazole]

A mixture of aromatic carboxylic acid (0.01mol) and thiosemicarbazone (0.01 mol) was irradiated in presence of magnesium sulphate (2 gm) for 5 minutes (250 watt). Obtained solid was filtered, dried, and recrystallized with suitable solvent.

Step 3 [Synthesis of mannich bases]

Various 2-amino-5-substituted-1,3,4-Thiadiazole [2a-2e] (0.01 mol) is added to a mixture of formaldehyde (0.9 ml) and 75% ethanol (15ml). The reaction mixture was stirred for 15 minutes at room temperature until it is dissolved. Then quinoxaline-2,3-dione [1a] (0.01mol) were added, then irradiated for 4 minutes at (250 watt). Obtained solid was filtered, dried, and recrystallized with suitable solvent.

The synthesized compounds were determined in open capillary tubes (Thermonilic melting point apparatus) and

were uncorrected. Thin layer chromatography was performed using precoated aluminum plates coated with silica gel GF254 [E. merk] of 0.25mm thickness, dichloromethane and methanol as the solvent system and UV chamber as the visualizing agent.

An IR spectrum was recorded on ABB BOMEM FTIR spectrometer using KBr pellets. 1H-NMR spectra of the compounds in DMSO was recorded on JEOL GSX 400 NMR spectrophotometers [21]

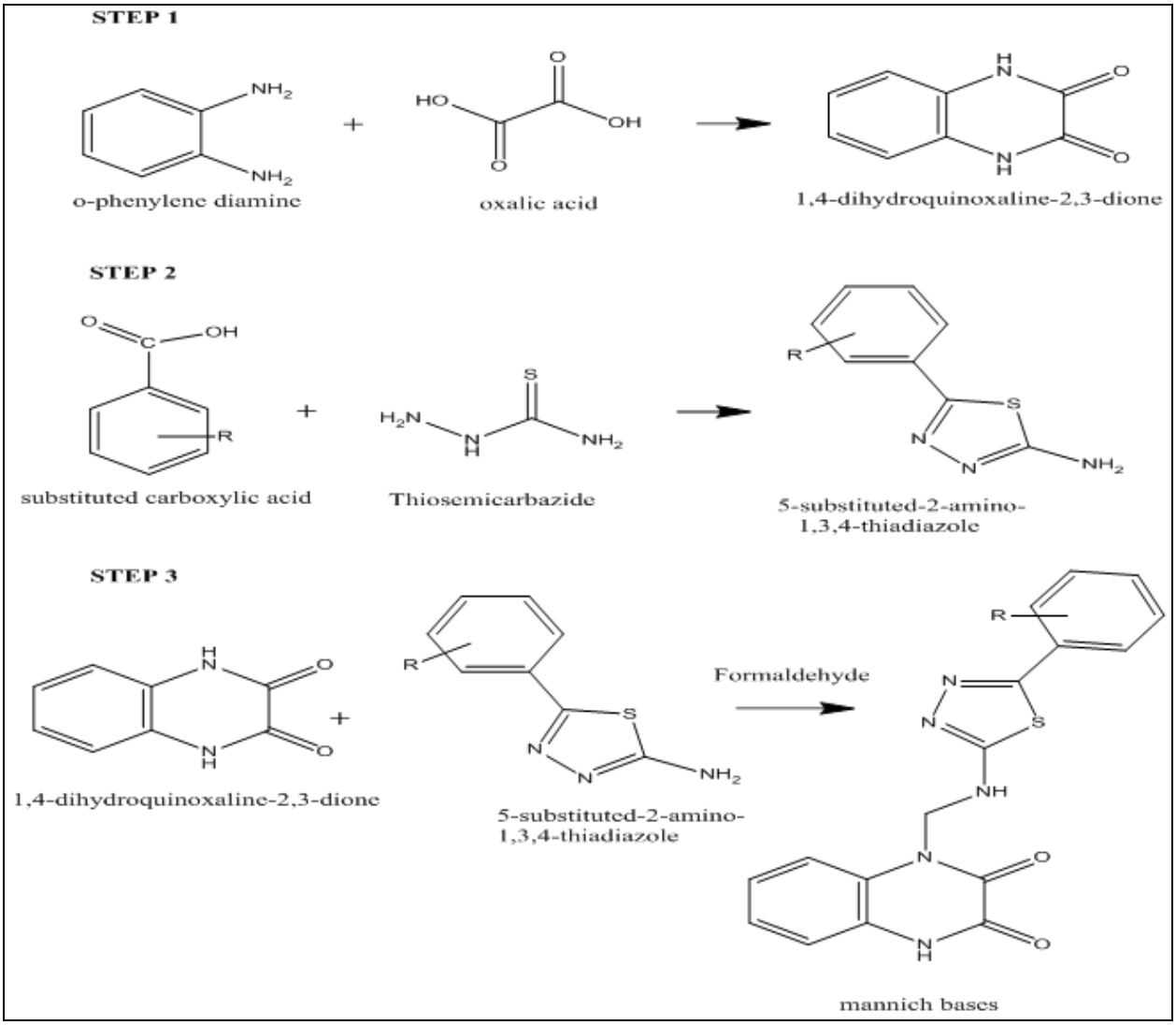
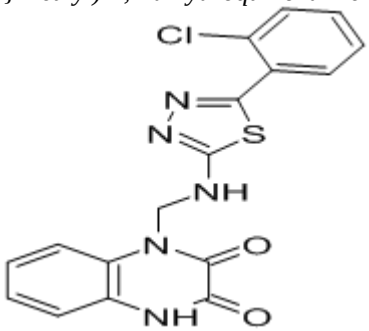
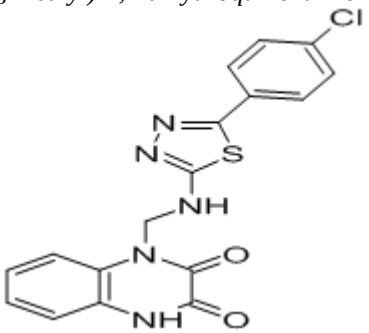
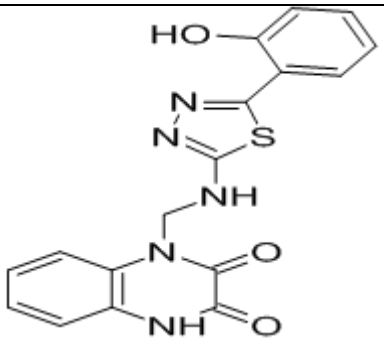
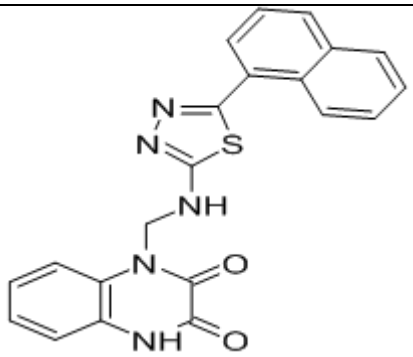


Fig 1: general scheme for synthesis of compounds

Table 1: Details of the R - represented in the synthetic scheme.

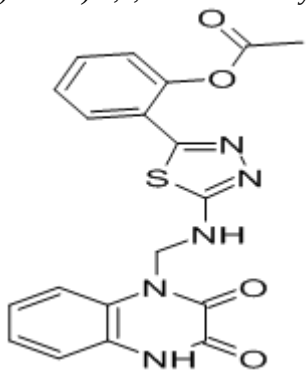
COMPOUND: 3a ***IUPAC NAME:*** *1-({[5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl] amino} methyl)-1,4-dihydroquinoxaline-2,3-dione* 	***COMPOUND: 3b*** ***IUPAC NAME:*** *1-({[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl] amino} methyl)-1,4-dihydroquinoxaline-2,3-dione.* 
COMPOUND: 3c ***IUPAC NAME:*** *1-({[5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl] amino} methyl)-1,4- dihydroquinoxaline-2,3-dione*	***COMPOUND: 3f*** ***IUPAC NAME:*** *1-({[5-(naphthalen-1-yl)-1,3,4-thiadiazol-2-yl] amino} methyl)-1,4-dihydroquinoxaline-2,3-dione*





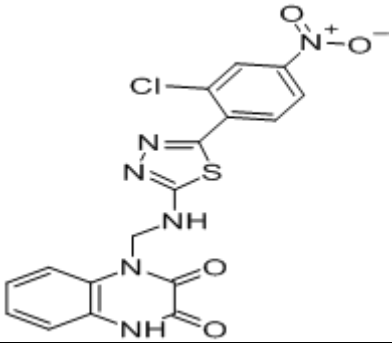
COMPOUND: 3d

IUPAC NAME: *2-(5-(((2,3-dioxo-3,4-dihydroquinoxalin-1(2H)-yl) methyl) amino)-1,3,4-thiadiazol-2-yl) phenyl acetate*



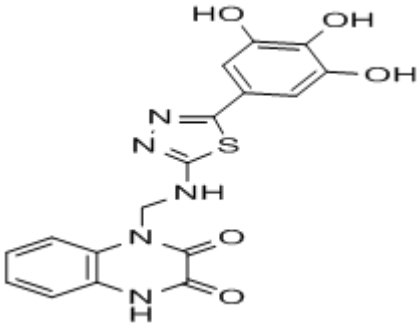
COMPOUND: 3g

IUPAC NAME: *1-(((5-(2-chloro-4-nitrophenyl)-1,3,4-thiadiazol-2-yl) amino) methyl)-1,4-dihydroquinoxaline-2,3-dione*



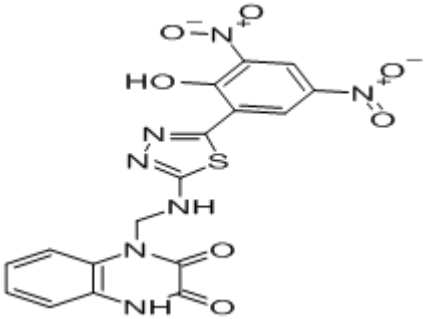
COMPOUND: 3e

IUPAC NAME: *1-(((5-(3,4,5-trihydroxyphenyl)-1,3,4-thiadiazol-2-yl) amino) methyl)-1,4-dihydroquinoxaline-2,3-dione*



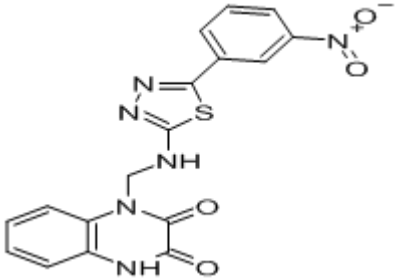
COMPOUND: 3h

IUPAC NAME: *1-(((5-(2-hydroxy-3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl) amino) methyl)-1,4-dihydroquinoxaline-2,3-dione*



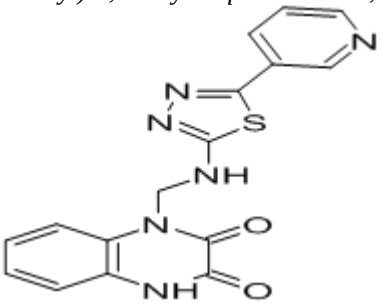
COMPOUND: 3i

IUPAC NAME: *1-(((5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl) amino) methyl)-1,4-dihydroquinoxaline-2,3-dione*



COMPOUND: 3l

IUPAC NAME: *1-({[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl] amino} methyl)-1,4-dihydroquinoxaline-2,3-dione*

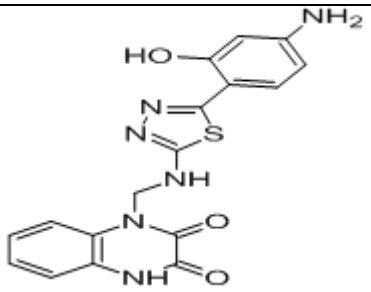


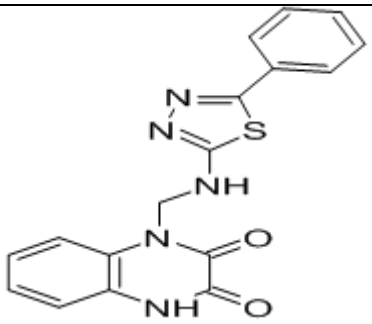
COMPOUND: 3j

IUPAC NAME: *1-(((5-(4-amino-2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl) amino) methyl)-1,4-dihydroquinoxaline-2,3-dione*

COMPOUND: 3m

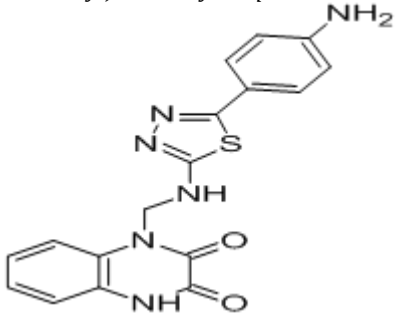
IUPAC NAME: *1-{[(5-phenyl-1,3,4-thiadiazol-2-yl) amino] methyl}-1,4-dihydroquinoxaline-2,3-dione*





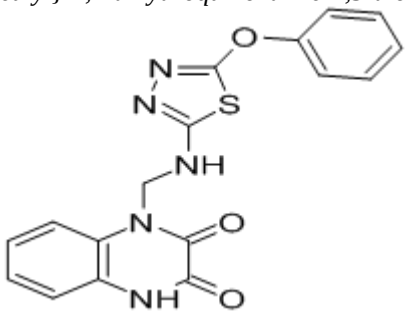
COMPOUND: 3k

IUPAC NAME: *1-({[5-(4-aminophenyl)-1,3,4-thiadiazol-2-yl] amino} methyl)-1,4-dihydroquinoxaline-2,3-dione*



COMPOUND: 3n

IUPAC NAME: *1-{[(5-phenoxy-1,3,4-thiadiazol-2-yl) amino] methyl}-1,4-dihydroquinoxaline-2,3-dione*



Animal studies

Evaluation studies of anti-inflammatory activity [22-29]

OECD guideline- 423

Evaluation Methods

Erythema assays

In this method, irradiation of shaven back skin of guinea pig with UV light causes erythema which can be reduced by anti-inflammatory agents.

Edema assays

The edema can be produced in experimental animals by the local injection of substance like, formaldehyde, carrageenan, histamine, dextron and ovalbumin.

Granuloma assays

There are two types of granuloma assays such as cotton wool pellet and granuloma pouch method.

Experimental arthritis assays

Poly arthritis induced in rats by injection of dead tubercle bacilli suspended in liquid paraffin is frequently used method. Kaolin, talc and even mercury have also been injected directly into joints of rats and pigeons to induce arthritis.

Experimental Protocol (Carrageenan induced paw oedema method in rats)

Anti-inflammatory activity was performed by carrageenan-induced paw oedema method in rats. Diclofenac sodium (20 mg/kg, i.p) was administered as standard drug for comparison. The synthesised compounds were administered at dose levels of (200 and 400 mg/kg) orally 30 min. prior to the administration of 0.1ml/kg body weight of carrageenan in saline (1% W/V) into the lateral malleolus of the sub-planter region of the left hind paw. The paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately before and 30 min, 1, 2 and 3 hr. after carrageenan injection. The percentage inhibition of paw odema was calculated by using the following formula and the results are depicted in Table - 5 and Table-6.

Percentage protection = [(Control-Test)/Control] X 100

Result and discussion

The structure of all newly synthesized 4-thiazolidinone derivatives of Quinoxaline2,3dione were confirmed on the basis of analytical and spectral data.

An IR spectrum was recorded on ABB BOMEM FTIR spectrometer using KBr pellets. 1H-NMR spectra of the compounds in DMSO was recorded on JEOL GSX 400 NMR spectrophotometers and animal studies as per standard procedures given in IP and OECD guidelines.

Synthesis with spectral data

Synthesis of 1-({[5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl] amino} methyl)-1,4-dihydroquinoxaline-2,3-dione [3a]

Brown solid; yield: 75.35%; FT-IR(KBr, cm-1): 3451.48(aromatic NH), 3419.35(aromatic CH), 3343.36(NH), 3232.14(aromatic CH), 2849.76(aromatic CH), 2587.72(C-S-C), 1633.44(C=O), 1557.42(aromatic C=C), 1319.91(C-N thiadiazole), 824.35(C-Cl), 739.35(C-H bending), 683.79 (C-H bending), 1HNMR (400MHz,DMSO):δ 2.509, 3.859, 4.114, 6.341, 6.373, 6.460, 6.527, 6.636, 6.644, 7.252, 7.304, 7.434, 7.440, 7.543, 7.591, 7.634, 7.679, 7.736, 7.772, 7.792, 8.287, 8.393, 8.445, M+ calculated for C17H12ClN5O2S is 385.8275 found 385.7109

Synthesis of 1-({[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl] amino} methyl)-1,4-dihydroquinoxaline-2,3-dione [3b]

Brick red solid; yield: 81.25%; FT-IR (KBr, cm-1): 3446.53(aromatic NH), 3343.27(NH), 3232.96(aromatic CH), 2854.49(aromatic CH), 2586.73(C-S-C),

1635.09(C=O), 1562.41(aromatic C=C), 1320.43(C-N thiadiazole), 824.26(C-Cl), 739.49(C-H bending), 677.42 (C-H bending), 1HNMR (400MHz, DMSO): δ 2.509, 3.859, 4.069, 6.550, 6.668, 6.675, 7.249. 7.562, 7.575, 7.635, 7.643. 7.683, 7.934, 7.953, 8.312, 8.428, 13CNMR: 166.96(C=O),130.11,129.22(C=N), 123.07,122.91,122.39(C=C), 116.99,115.64,113.84 (ArC), 40.32(C-Cl), 39.90,39.69,39.48(C), 33.56(CH), 31.34(CH2), M+ calculated for C17H12ClN5O2S is 385.8275 found 385.7216

Synthesis of 1-({[5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl] amino} methyl)-1,4- dihydroquinoxaline-2,3-dione [3c]

Greyish solid; yield: 72.75%; FT-IR(KBr, cm-1): 3444.06(aromatic NH), 3345.03(NH), 3231.62(aromatic OH), 2851.84(aromatic CH), 2589.16(C-S-C), 1634.79(C=O), 1558.76(aromatic C=C), 1320.25(C-N thiadiazole), 1119.01(C-O), 739.02(C-H bending), 614.34 (C-H bending), 1HNMR (400MHz,DMSO):δ 2.506, 3.433, 3.680, 3.741, 3.857, 3.935, 3.959, 4.073, 6.341, 6.371, 6.544, 6.659, 6.894, 6.992, 7.023, 7.249, 7.255, 7.307, 7.461, 7.595, 7.628, 7.636, 7.678, 7.721, 7.784, 8.011, 8.302, 8.410, 9.621, 11.282, M+ calculated for C17H13N5O3S is 367.3818 found 367.1017

Synthesis of 2-(5-(((2,3-dioxo-3,4-dihydroquinoxalin-1(2H)-yl) methyl) amino)-1,3,4-thiadiazol-2-yl) phenyl acetate [3d]

Greyish solid; yield: 78.25%; FT-IR(KBr, cm-1): 3441.59(aromatic NH), 3343.59(NH), 3231.62(aromatic OH), 3232.42(aromatic CH), 2859.40(aromatic CH), 2591.23(C-S-C), 1659.52(C=O), 1634.91(C=O), 1562.70(aromatic C=C), 1320.51(C-N thiadiazole), 1065.13(C-O), 739.29(C-H bending), 612.13 (C-H bending), 1HNMR (400MHz,DMSO):δ 2.508, 3.395, 3.681, 3.850, 3.922, 4.072, 6.344, 6.371, 6.506, 6.616, 6.620, 6.870, 7.242, 7.276, 7.293, 7.584, 7.617, 7.625, 7.669, 7.729, 7.781, 8.259, 8.347, M+ calculated for C19H15N5O4S is 409.4185 found 409.4508.

Synthesis of 1-(((5-(3,4,5-trihydroxyphenyl)-1,3,4-thiadiazol-2-yl) amino) methyl)-1,4-dihydroquinoxaline-2,3-dione [3e]

Dark greyish solid; yield: 60.45%; FT-IR(KBr, cm-1): 3444.06(aromatic OH), 3352.64(NH), 3231.57(aromatic OH), 2856.01(aromatic CH), 2591.63(C-S-C), 1634.78(C=O), 1566.23(aromatic C=C), 1321.62(C-N thiadiazole), 1070.47(C-O), 740.97(C-H bending), 611.33(C-H bending), 1HNMR (400MHz,DMSO):δ 2.506, 3.858, 4.073, 4.102, 6.526, 6.533, 6.542, 6.654, 6.661, 6.923, 7.249, 7.297, 7.307, 7.601, 7.629, 7.634, 7.676, 7.716, 8.283, 8.312, 8.375, 8.431, M+ calculated for C17H13N5O5S is 399.3806 found 399.3376.

The anti-inflammatory activity of the synthesized compounds was evaluated by Carrangeenan induced paw oedema method in rats. The activity was studied at 200 and 400 mg/kg b.w., p.o. and their effects were measured at 30, 60, 120 and 180 min.

Most of the synthesized compounds exhibited moderate to good anti-inflammatory activity. All the synthesized compounds were exhibited highest activity at 120 min.

Table 2

Compound	Dose (mg/kg)	30 min		60 min		120 min		180 min	
		Mean±SEM	%	Mean±SEM	%	Mean±SEM	%	Mean±SEM	%
3a	400	0.44±0.01	36.60	0.50±0.01	41.85	0.67±0.01	52.48	0.57±0.01	43.56
3b	400	0.46±0.01	33.81	0.53±0.02	37.20	0.69±0.01**	51.06	0.58±0.01	42.57
3c	400	0.57±0.02	18.32	0.61±0.01	27.90	0.87±0.01	32.29	0.65±0.01	35.64
3d	400	0.53±0.01*	23.96	0.59±0.02*	31.38	0.86±0.02	39.00	0.66±0.02*	34.65
3e	400	0.60±0.01*	15.50	0.61±0.01**	26.06	0.81±0.01*	42.55	0.64±0.01**	35.57
DiclofenacSodium(Standard)	20	0.39±0.001**	46.48	0.35±0.01**	59.31	0.31±0.01	78.01	0.39±0.05*	61.38
Control	-	0.72±0.01**	-	0.86±0.01**	-	1.41±0.04*	-	1.01±0.01*	-

Significant differences with respect to control was evaluated by ANOVA, Dunnet's test *P<0.05, **P<0.01

When compared to standard drug (Diclofenac sodium 20 mg/kgi.p), compounds 3a and 3b were found to exhibit good anti-inflammatory activity. Compounds 3c,3d and 3e were found to exhibit moderate activity.

The anti-inflammatory activity shown by the synthesized compounds were 3b>3a>3d>3c>3e.

Compound 3b having Chloro group at para position of phenyl ring enhances the anti-inflammatory activity, which might serve as new templates in the synthesis and development of potent therapeutics.

From the anti-inflammatory activity evaluation, it is very clear that the tested compounds showed near to equipotent activity to that of standard diclofenac sodium employed for the study. From antiinflammatory activity evaluations, it was found that compounds showed significant activity. Perhaps the presence of Chloro group at aromatic system and presence of heterocyclic nucleus may be responsible for marked anti-inflammatory activity.

Acknowledgement

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Conflict of interest

No conflict of interest

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