



Synthesis, characterization and pharmacological evaluation of some potent 2-(substituted phenylimino) quinoxaline-3-one for their analgesic activity

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ABSTRACT

A series of Schiff bases such as 2-(substituted phenylimino) quinoxaline-3-one were synthesized by nucleophilic addition reaction of quinoxaline-2,3-dione with *p*-Phenylene diamine followed by the Schiff reaction using different aldehydes in alcohol. A total of five compounds were synthesized by both conventional and microwave oven method with good percentage yield. They were purified and characterized as the basis of their spectral data. The synthesized compounds were screened for *in-vivo* analgesic activity at a dose of 20 mg/kg body weight. All the compounds showed significant analgesic activity comparable with control and standard drug aspirin in rats described in eddy's hot plate method. Among them, compounds 3e and 3d exhibited potent analgesic activity. Effect of substitution at arylidene part on analgesic activity was studied. Compounds 3e and 3d with electron withdrawing groups were found to be highly potent among the series.

Key words: Quinoxaline-2, 3-Dione, Schiff base, 2-(substituted phenylimino) quinoxaline-3-one, analgesic activity, substituent.

INTRODUCTION

Pain is not easy or satisfactorily defined and, therefore, is often interpreted as a suffering that results from the perception of painful stimuli. It's a common symptom and it indicates that something is wrong in the body and may give a clue to the nature of the disease. Hence, "pain is a specific sensation with its own peripheral and central mechanisms independent of other five senses." Pain itself is not a disease; it is by far the most common medical complaint. It is usually perceived as an indication of ill health and most diseases have a component of pain. The control of pain is one of the most important uses to which drugs are put. Pain can be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain. Drugs, which alter the pain sensitivity or remove pain, are called as painkiller or analgesics [1, 2].

Relief of pain and inflammation in the human being is a major challenge for medicinal chemistry researchers. Drug discovery and development continue to be a challenge, with increasingly high investments in R&D and increased numbers of submissions failing to translate into the delivery of novel chemical entities onto the market. Clinical treatment of pain today is dominated by two main groups of analgesics. The wide availability of generic and over-the-counter analgesics based on non-steroidal anti-inflammatory (NSAIDs), acetaminophen, and "weak" opiates

(and their combinations) provides many individuals with an accessible source of relief for mild to moderate pain [3,4]. Given the reluctance to use opiates because of their liability towards physical dependence, tolerance, respiratory depression and constipation, and the limitations in the efficacy of the peripheral analgesics associated to classical drawbacks i.e. Gastrointestinal irritation and lesions, renal toxicity and inhibition of platelet aggregation, while the use of opioid is limited to severe pain because of adverse secondary reactions as respiratory depression, dependence, sedation [5,6] and the quest are to develop new potent analgesic agents with the efficacy of morphine without the undesired side effects and however, many patients with chronic conditions such as osteoarthritis remain poorly treated [3,4].

Quinoxaline (benzopyrazines), derivatives are compounds containing a ring complex made up of a benzene ring and a pyrazine ring; they are isomeric with the cinnolenes, phthalazines and quinazolines [5]. The synthesis and chemistry of quinoxalines have attracted considerable attention in the past ten years. Some of them exhibit biological activities including analgesic [6], antiviral, antibacterial, anti-inflammatory, anticancer, anti-depressant, anti-HIV and as kinase inhibitors [7].

Schiff bases are important classes of ligands that coordinate with metal ions via azomethine nitrogen and have been studied extensively because of increasing recognition of their role in the biological system [8, 9]. Schiff bases are used as substrates in the preparation of a number of biologically active compounds. Moreover, Schiff bases derived from various heterocycles have been reported to possess anti-fungal, anti-cancer, cytotoxic, and anti-convulsant activities [10, 11].

The already existed Schiff bases containing quinoxaline moieties such as 4-(2-methylquinoxalinyloxy) benzaldehyde, 2-[4-(substituted-benziminomethyl) -phenoxy] -3-methyl quinoxalines, 4-(2-methyl-quinoxaline-3-yloxy) benzamine and 4-(2-methylquinoxalin-3-yloxy) -N-substituted benzylidene benzamines were tested for their antimicrobial activity [12]. The literature survey reveals that not much work has been carried out on the synthesis and evaluation of the analgesic activity of Schiff base ligand derived from Quinoxaline -2, 3-Dione. In view of the above findings and in continuation of research work on synthesis and characterization of new Schiff base ligands and their pharmacological evaluation towards analgesic activity.

EXPERIMENTAL SECTION

The reagent grade chemicals were obtained from commercial sources and purified by either distillation or recrystallization before use. Microwave reactions were carried out in a domestic microwave oven (Samsung model) Purity of synthesized compounds has been checked by thin layer chromatography. Melting points were determined by Veego VMP-1 the melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR spectrometer model using KBr discs. The NMR spectra (DMSO-d₆) were recorded on Bruker DRX-300 spectrometer with TMS as an internal standard. The mass spectra were measured on a Shimadzu LCMS 2010A spectrometer

General procedure for the synthesis of Schiff base of quinoxaline-2, 3- Dione (3a -3e) by conventional method

Step 1: A powdered mixture of oxalic acid dihydrate (0.01 mol, 1.26 g) and o-phenylene diamine (0.01 mol, 1.0814 g) were refluxed on an oil bath for 90 minutes and cooled. The crystals separated was filtered, washed with water and crystallized with 5% NaOH/Dil HCl. Synthesis of quinoxaline-2, 3-dione (compound 1) was identified by thin layer chromatography (TLC) in Chloroform: Methanol (9:1) [13].

Step 2: Equimolar quantities of (0.01 mol) quinoxaline-2, 3-dione and p-phenylene diamine were dissolved in a sufficient quantity of methanol (30 ml) in the presence of acetic acid and refluxed for one hour, and then kept for two hours at room temperature (37°C). Compound 2 was separated out, filtered, dried in a vacuum and recrystallized from absolute ethanol. Equimolar quantities (0.01 mol) of compound 2 and various aldehydes were dissolved in ethanol containing a catalytic amount of glacial acetic acid and refluxed for eight hours. After standing for one-to-two days at room temperature, the product of different Schiff bases of quinoxaline -2, 3 -Dione such as 2-(substituted phenylamino) quinoxaline-3-one (Compound 3a-3e) separated out which was filtered, dried, and recrystallized from absolute ethanol (8) Progress of the reaction was checked with TLC.

Microwave synthesis of Schiff bases of quinoxaline-2, 3- Dione (3a -3e)

Step 1: A powdered mixture of oxalic acid dihydrate (0.01 mol, 1.26 g) and O-phenylene diamine (0.01 mol, 1.0814 g) was taken in an open beaker and 1 ml of water added and mixed thoroughly. The mixture was irradiated in a domestic microwave oven irradiation (MWI) at an emitted power of 400 Watt (W) for 3 mins. 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 to afford colorless needles. The needles obtained was filtered, washed with water and crystallized with 5% NaOH/ Dil HCl. Quinoxaline-2, 3-dione was identified by TLC in Chloroform: Methanol (9:1).

Step 2: Equimolar quantities of (0.01 mol) quinoxaline-2, 3-dione and p-phenylene diamine were dissolved in a sufficient quantity of methanol (30 mL) in the presence of acetic acid and this mixture was irradiated in microwave at 400 W for 7 mins. Compound 2 was separated out, filtered, dried in a vacuum, and recrystallized from absolute ethanol. In a hard glass tube, (0.01 mol) compound 2 and Substituted aromatic aldehyde (0.01mol), few drops of acetic acid were taken and mixed well to prepare a paste. This mixture was irradiated in the microwave at 450 watts till the completion of the reaction. The progress of the reaction was checked with TLC (hexane-ethyl acetate 8:2). After the completion of the reaction, the reaction mixture was cooled to room temperature and ice-cold water was added to it. It was filtered, washed with water and purified by recrystallization through glacial acetic acid.

Characterization

The all synthesized compounds are characterized by molecular weight, melting point, percentage yield, solubility, R_f values, Infrared, Mass, ¹³C NMR and ¹H NMR spectroscopy.

Quinoxaline- 2, 3-Dione

Colorless crystals (82 % CM, 88% MW); mp >340 0C; R_f (chloroform/methanol 9:1) 0.52; IR (KBr, cm-1): 1700 & 1681 (C=O str, ketone), 1247 (C-N str), 3344 (N-H str), 854 (Ar C-H bend), 3050 (Ar C-H str), 1593 (C=C str); ¹H-NMR (DMSO-d₆) δ: 11.7 (s, 2H, NH), 7 (m, 4H, Ar-H); ¹³C (DMSO-d₆) δ: 164.92 (C-2), 163.11 (C-3), 137.10 (C-4a & C-8a), 116.47 (C-5 & C-8), 128.54 (C-7), 123.45 (C-6); MS (m/z): M+ calculated 162.15, found 161.98

2-(4-ethylideneamino) phenylimino) quinoxaline-3-one (3a)

White crystal (70% CM, 80% MW); mp 300-310; IR(KBr, cm-1):1681 (C=O str), 1597 (C=N), 911 (Ar C-H bend), 3150 (Ar C-H str),1582(C=C str), 1199 (C-N str); ¹H-NMR (DMSO-d₆) δ: 12.1 (d, 2H, NH), 7 (m, 8H, Ar-H), 8.507 (s, 1H, -CH=N-), 2.48 (s, 3H, CH₃); ¹³C (DMSO-d₆) δ: 165.11 (C2 =O), 167.27 (C3=N), 129.10 (C-4a & C-8a), 116.47 (C-5 & C-8), 128.54 (C-7), 123.45 (C-6), 160.3 & 151.6 (-N=CH- in C-1' and C-4'), 130.4- (C-2' & C-6'), 122.1 (C-3' & C-5') 10.6 (CH₃); MS (m/z): M+ calculated 278.28 found 278.85.

2-(4-(benzylideneamino) phenylimino) quinoxaline-3-one (3b)

White crystal (68.5% CM, 73% MW); mp >300; IR(KBr, cm-1):1701 (C=O str), 1532 (C=N), 917 (Ar C-H bend), 3100 (Ar C-H str),1575(C=C str), 1203 (C-N str); ¹H-NMR (DMSO-d₆) δ: 10.8 (d, 2H, NH), 6.2- 7.5 (m, 13H, Ar-H), 8.32 (s, 1H, -CH=N-); ¹³C (DMSO-d₆) δ: 164.01 (C2 =O), 162.3 (C3=N), 127.2 (C-4a & C-8a), 120.4 (C-5 & C-8), 125.35 (C-7), 120.5 (C-6), 161.5 & 154.2 (-N=CH- in C-1' and C-4'), 130.9 (C-2' & C-6'), 122.8 (C-3' & C-5'), 133.4(C-1"), 128.5 – 129.2 (C-2", 3", 4", 5", 6"), 161.5 (C-9=N) ; MS (m/z): M+ calculated 340.34 found 340.85

2-(4-(4-hydroxybenzylideneamino) phenylimino) quinoxaline -3-one (3c)

Yellowish white crystal (71.4% CM, 75.5% MW); mp >300; IR(KBr, cm-1):17101 (C=O str), 1521 (C=N), 3438 (Ar-OH str) 929 (Ar C-H bend), 3011 (Ar C-H str),1575(C=C str), 1210 (C-N str); ¹H-NMR (DMSO-d₆) δ: 11.9 (d, 2H, NH), 6.5- 7.5 (m, 12H, Ar-H), 8.23 (s, 1H, -CH=N-), 5.14 (s, 1H, Ar-OH); ¹³C (DMSO-d₆) δ: 162.81 (C2 =O), 160.1 (C3=N), 129.52 (C-4a & C-8a), 127.5 (C-5 & C-8), 119.45 (C-7), 120.5 (C-6), 159.5 & 151.6 (-N=CH- in C-1' and C-4'), 130.7 (C-2' & C-6'), 121.9 (C-3' & C-5'), 128.4(C-1"), 117.5 (C-2" & 5"), 130.1 (C-3" & 6"), 160.7 (C4" – OH), 160.1 (C-9=N) ; MS (m/z): M+ calculated 357.34 found 357.10

2-(4-(4-chlorobenzylideneamino) phenylimino) quinoxaline -3-one (3d)

White crystal (67% CM, 73% MW); mp >300; IR(KBr, cm-1):1711 (C=O str), 1587 (C=N), 643 (Ar-Cl str), 947 (Ar C-H bend), 3028 (Ar C-H str),1485(C=C str), 1219 (C-N str); ¹H-NMR (DMSO-d₆) δ: 12.4 (d, 2H, NH), 6.5- 7.5 (m, 12H, Ar-H), 8.4 (s, 1H, -CH=N-); ¹³C (DMSO-d₆) δ: 164.2 (C2 =O), 175.1 (C3=N), 133.2 (C-4a & C-8a), 125.5 (C-5 & C-8), 121.45 (C-7), 119.5 (C-6), 154.3 & 157.4 (-N=CH- in C-1' and C-4'), 131.2 (C-2' & C-6'), 128.9 (C-3' & C-5'), 128.4(C-1"), 117.5 (C-2" & 5"), 130.1 (C-3" & 6"), 135.7 (C4" – Cl), 160.4 (C-9=N) ; MS (m/z): M+ calculated 373.58 found 373.47

2-(4-(4-nitrobenzylideneamino) phenylimino) quinoxaline -3-one (3e)

Yellow crystal (82% CM, 85% MW); mp >300; IR(KBr, cm⁻¹):1719 (C=O str), 1601 (C=N), 671 (Ar-Cl str), 953 (Ar C-H bend), 3110 (Ar C-H str),1499(C=C str), 1201 (C-N str); ¹H-NMR (DMSO-d₆) δ: 11.7 (d, 2H, NH),6.5-7.5 (m, 12H, Ar-H), 8.2 (s, 1H, -CH=N-); ¹³C (DMSO-d₆) δ: 161.2 (C2 =O), 168.4 (C3=N)), 132.8 (C-4a & C-8a), 126.5 (C-5 & C-8), 121 (C-7), 120.1 (C-6), 152.7 & 155.3 (-N=CH- in C-1' and C-4'),130.9 (C-2'& C-6'),128.5 (C-3' & C-5'), 129.1(C-1"),119.5 (C-2"&5"),131 (C-3"&6"), 150.9 (C4" -NO₂), 160.9 (C-9=N) ; MS (m/z): M+ calculated 384.12 found 385.01

Pharmacological Evaluation

Healthy, adult Wister rats of both sex male: female (1: 1) weighing 120–140g was used which was approved by the Institutional animal ethics committee and were maintained in individual polypropylene cages, with free access to ration and water. The animals left for two days for acclimatization to animal room conditions and were maintained on a standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but allowed free access of water. Test samples and reference compounds were suspended in 0.5% carboxymethyl cellulose and administered to each rat by using gastric gavage needle. The control group animals, however, received the same volume of dosing vehicle. In the pharmacological studies, the animals were first administered in 20 mg/kg (body weight) dose of the Test drugs.

Acute Toxicity Studies

The Acute toxicity test was performed for the entirely synthesized compound to ascertain the LD₅₀ values as per OECD guidelines. The experimental dose was selected between the minimum effective dose and maximal non-lethal dose. The dose level up to 100 mg/kg of the synthesized hydrazones in Wister rats were not produced any mortality on oral administration. So 20 mg/kg doses of Schiff bases of quinoxaline 2, 3-dione was fixed for this study.

In-Vivo Analgesic activity by Eddy's Hot Plate Method

Heat is used as a source of pain. Animals were individually placed on the hot plate maintain at a constant temperature (55°C) and the reaction of animals, such as paw licking or jump off was taken as the end response. Analgesic drugs/compounds increase the reaction time. The method was first described by Eddy & Leimbach (A cut-off period of 15 Sec is observed to avoid damage to the paw). Administration of the control, standard and test compounds to animals by i.p route and note the reaction of time of animals at 10, 20, 30, 40 & 50 min interval on the hot plate after 60 min of drug administration. A group of rats were treated orally with a dose of 20 mg/kg BW with the aqueous suspension in 0.5% CMC Na of the synthesized compounds. The method of Eddy and Leimbach using techno heated plat analgesic apparatus was used [14, 15]. The standard drug aspirin (50mg/kg) was used reference drug for comparison. The result was tabulated in Table 1

Statistical Analysis

Results were expressed as means ± S.E.M. Statistical significance was analyzed using the one-way analysis of variance followed by Tukey's Multiple Comparison Test.

RESULTS AND DISCUSSION

Chemistry

The synthetic route followed for obtaining Schiff bases of quinoxaline-2,3-dione by conventional and microwave irradiation (3a–e) are outlined in Scheme. Thus, cyclo condensation of the o-phenylene diamine with oxalic acid in the presence of hydrochloric acid afforded quinoxaline- 2, 3-dione (compound 1). The percentage yields of the synthesized compounds were good. The compound 1 were reacted with p- Phenylenediamine by nucleophilic addition reaction through the carbonyl group in quinoxaline-2,3-Dione with NH₂ group in P- Phenylenediamine. The formed compound was undergone Schiff reaction with different aldehydes and the percentage yield was 70–90%. The synthesized compounds were checked for their purity through melting point determination and TLC. Further, all the compounds were characterized by spectral analysis such as IR, ¹H & ¹³C NMR, and mass spectra. The data are consistent with the assigned structures. From the compound o-phenylene diamine, compound -1 was synthesized. It was confirmed by the presence of peaks for ketone C=O str at 1708.9 cm⁻¹ , C-N str at 1247.9 cm⁻¹ in IR spectra and the presence of N-H peak at 12, Ar C-H peak at 7.1 ppm in ¹H NMR and . The compound 1 was used to prepare 2-(substituted phenylimino) quinoxaline-3-one. This is confirmed by the presence of C=N at 1597-1601 and absence of one ketone C=O in IR and the presence of -CH=N- peak at 8.0-8.4 ppm in ¹H NMR. ¹³ C-NMR showed characteristic signal for carbons of -N=CH- in C-1' and C-4' at 152.7 & 155.3 ppm. In this way our study based on

the synthesis of various substituted Schiff bases of quinoxaline-2, 3- dione(s) and evaluated for their In-vivo analgesic activity.

Analgesic activity

The Analgesic activity of all 2-(substituted phenylimino) quinoxaline-3-one (s) was evaluated using Hot plate which was displayed in table 1. As from the tables, it could be seen that all compounds showed significant analgesic activity comparable to the control and reference drug. The substitution with the different substituent on the phenyl of the aldehydic group of quinoxaline-2, 3-dione moiety plays an important role in the protection of the algesia. The compounds 3d & 3e are favorable for enhancing the analgesic activity of the compound. Thus, the degree of potency in ascending order was 3a to 3e. The result favors and proved that different substituted aldehyde compound plays an important role in an analgesic activity. Out of five newly synthesized derivatives compound 3e (p-NO₂) bearing strongly deactivating group at the para position of benzylidene ring was found to be very potent analgesic activity when compared with the standard drug aspirin. Whereas, methyl substituted Schiff base derivative (3a) showed reduced activity than other synthesized compounds. These results indicated that electron-donating group and the lack of benzene ring could lead the decrease of activity significantly. The compound 3c showed moderate activity than all the synthesized compounds. This revealed the presence of hydroxyl groups on the benzene ring as strong activating electron donating nature for the moderate activity.

Table 1: Evaluation of In-vivo Analgesic activity of the synthesized Schiff bases of quinoxaline-2,3-dione (3a-3e) by Eddy's Hot Plate Method in rats

| Compounds | Reaction time in minutes | | | | | |
|-----------|--------------------------|-------------|-------------|-------------|--------------|-------------|
| | 10 | 20 | 30 | 60 | 90 | 120 |
| Control | 3.42±0.03 | 3.48±0.02 | 3.64±0.04 | 3.50±0.03 | 3.76±0.04 | 3.64±0.05 |
| standard | 8.50 ±0.01 | 10.43± 0.01 | 11.29±0.01 | 11.61±0.01 | 12.50±0.01 | 12.90±0.01 |
| 3a | 5.71± 0.01 | 8.54±0.01 | 9.11± 0.01 | 10.24± 0.01 | 10.62± 0.01 | 10.87± 0.01 |
| 3b | 6.84±0.01 | 8.65±0.01 | 9.98±0.01 | 10.5±0.01 | 10.88±0.01 | 11.10±0.01 |
| 3c | 6.80 ± 0.15 | 9.01 ± 0.01 | 9.63±0.15 | 11.13±0.21 | 10.95± 0.01 | 11.14± 0.01 |
| 3d | 8.33± 0.02 | 10.93±0.02 | 11.12± 0.02 | 11.59 ±0.02 | 11.84 ± 0.01 | 12.14 ±0.01 |
| 3e | 8.57±0.01 | 10.15±0.01 | 11.43±0.01 | 11.71±0.01 | 12.30±0.01 | 12.98±0.01 |

P < 0.001 compared with control

CONCLUSION

In an attempt of developing a new class of analgesic, a new series of Schiff bases of quinoxaline-2, 3-dione derivatives efficiently synthesized with good yield. The analgesic activity study revealed that some derivatives came out to be very good analgesic. Compounds 3d and 3e emerged as excellent analgesic agents. Structural activity relationship revealed that incorporation of electron withdrawing groups at p-position on benzylidene ring enhanced the activity. Further investigations in the future should determine analgesic and anti-inflammatory potency of title compounds, the effect of specific substituent and their positions in the molecules, and their related toxicity. So they might serve as lead molecules to obtain more clinically useful, novel entities in the future.

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