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Synthesis of certain quinoxaline derivatives of expected antiinflammatory and analgesic activities

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ABSTRACT

This study involves a survey covering the synthesis, reaction and biological values of some quinoxaline derivatives. Combination of quinoxaline nucleus with certain pharmacologically active compounds (antheranilic, 4-amino benzoic, salicylic, 4-amino salicylic, 5-amino salicylic acids, *2*-amino phenol and 4-aminophenol)[1] which were known to have anti-inflammatory and analgesic activities was the aim of this study to give a novel target compounds expected to have anti-inflammatory and analgesic activities. This was accomplished during the coarse of this thesis via three schemes each comprises the incorporation of quinoxaline nucleus with certain pharmacologically active nuclei knowing to have anti-inflammatory and analgesic activities.

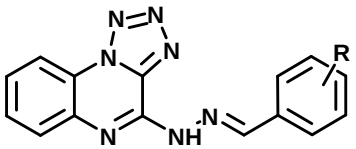
KEYWORDS

Quinoxaline;
Anti-inflammatory;
Analgesic.

INTRODUCTION

Many quinoxalines have anti-inflammatory and analgesic activities as hydrazone derivative of quinoxaline (1)[1] and tetrazoloquinoxalines (2).[2]

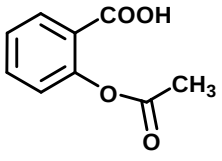
(1)



(2)

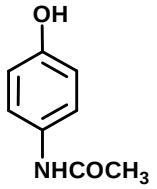
R= 2-OH, 3-OH, 4-OH, 2-Cl or 3-Cl

Anti-inflammatory activity of non steroidal anti-inflammatory drugs (NSAID) including salicylates (5)[3-5], *N*-arylanthranilic acids (6)[6-8] and *p*-aminophenol derivatives (7)[9] were reported.



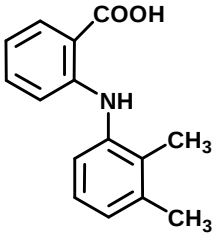
Acetylsalicylic acid

(5)



Paracetamol

(6)



Mefenamic acid

(7)

Hence, it is very interesting to combine NSAID moieties and quinoxaline nucleus aiming to obtain more

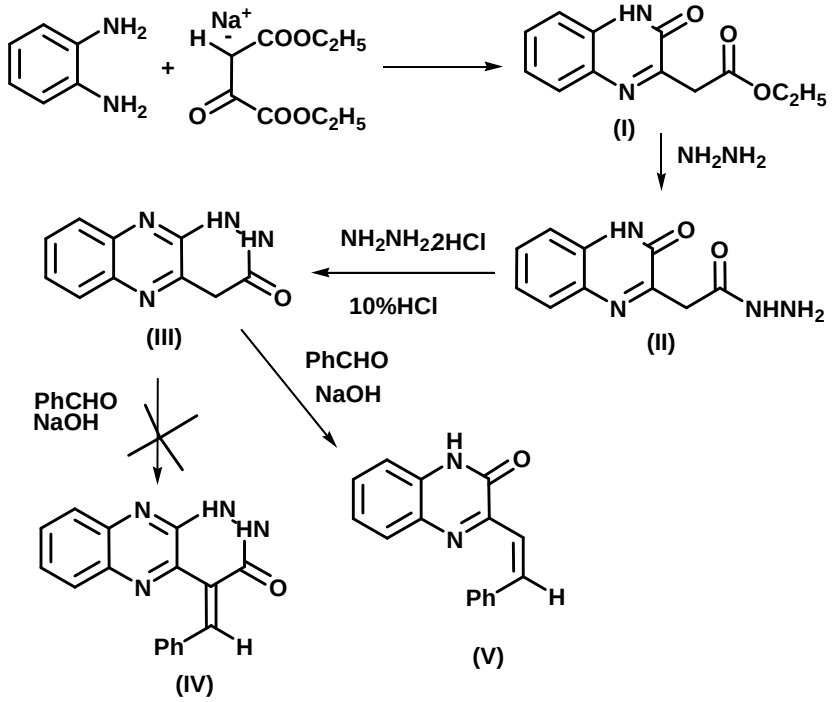
effective and less toxic anti-inflammatory analgesic compounds.

Some novel azo derivatives of 3,5-dimethyl-1-(2-hydroxyethyl)pyrazole were synthesized and they had shown potent analgesic activity.[10]

CHEMISTRY

Scheme I includes the reaction of *o*-phenylene diamine with sodium salt of ethyl oxalacetate to obtain ethyl 3-oxo-3,4-dihydroquinoxaline-2-yl acetate (I)[11] which upon hydrazinolysis give 2-[3-oxo-3,4-dihydroquinoxalin-2-yl]acetohydrazide (II)[12] that underwent cyclization to give 1,4-dihydropyridazino[3,4-b]quinoxalin-3(2H)-one (III)[13].

Condensation of the later with benzaldehyde afforded the 3-(2-phenylethenyl)quinoxalin-2(1H)-ones (V).



Scheme 1

Scheme II consisted of coupling of I with diazonium salts of antheranilic, 4-amino benzoic acids, *o*- and *p*-toludenes and anisidenes to afford differet, new azocompounds, syn and anti ethyl 2-(arylhydrazin-1-ylidene)-2-(3-oxo-3,4-dihydroquinoxalin-2-yl)acetates VIa-f.

Also scheme II includes hydrolysis of I with potassium hydroxide followed by acidification give 3-Methylquinoxalin-2(1H)-one (VII)[14] which also coupled with diazonium salts of phenols, *p*-anisidine and salicylates to afford different new azocompunds 3-[(2-substituted hydrazin-1-ylidene)methyl]quinoxalin-2(1H)-ones VIIIa-e.

Scheme III consists of subjecting of VII to mannich reaction with different primary amines to give 3-[2-(substituted phenylaminoethyl)]quinoxalin-2-(1H)-ones IXa-c.

Chlorination of VII gave 2-chloro-3-methylquinoxalines (X) which upon further nucleophilic substitution of chloride with different phenols, salicylates, antheranilic and 4-aminobenzoic acid gave 2-substituted-3-methylquinoxalines XIa-e.

Screening of some of the final compounds for their anti-inflammatory and analgesic activities, was carried out and some of them showed both activities together.

RESULT AND DISCUSION

Chemistry

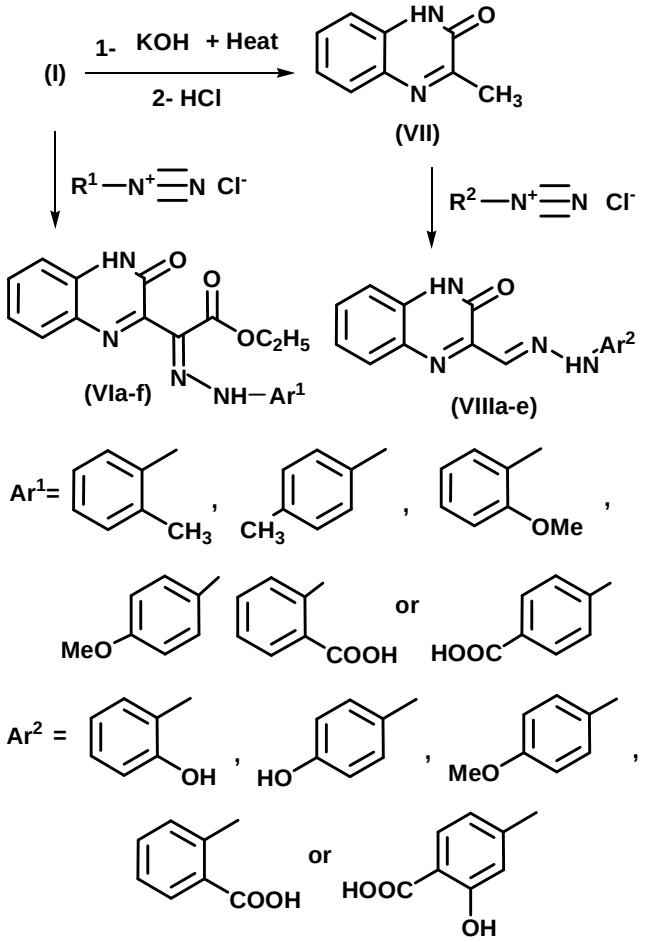
Compound **(I)** was reported to be prepared in two steps via condensation of diethyl oxalate with ethyl acetate in benzene containing sodium metal to form the sodium salt of ethyl oxalacetate, which was condensed

with *o*-phenylenediamine in acetic acid to give compound **(I)**, in yield of 80%[11].

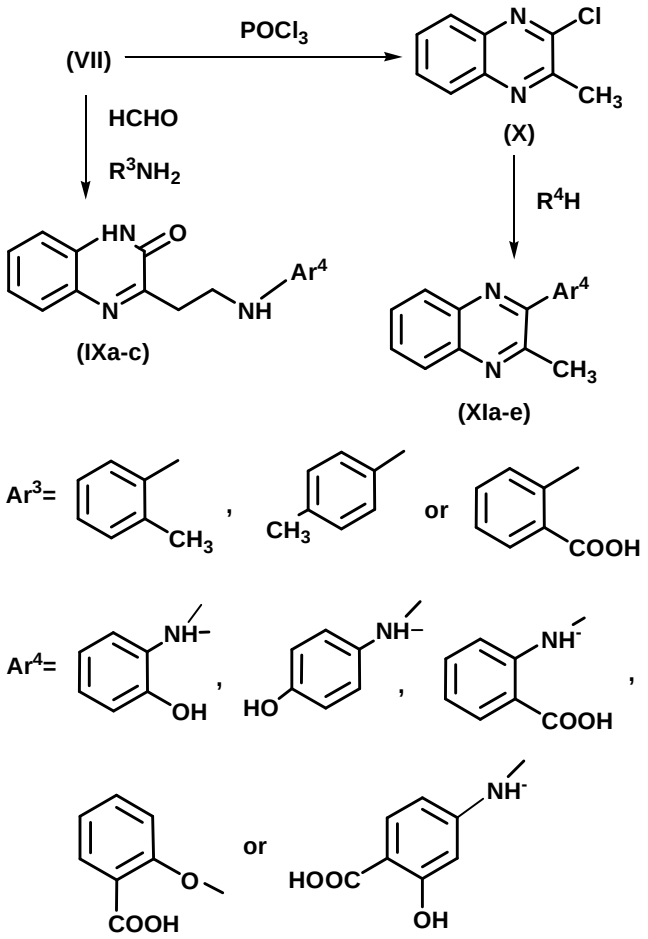
In this work, sodium ethoxide / ethanol was used instead of sodium metal / benzene. This modification resulted in a higher yield (88%). Compound **(II)** was first prepared in 1983[12] by hydrazinolysis of ethyl 3-oxo-3,4-dihydroquinoxalin-2-yl-acetate **(I)** with hydrazine hydrate in absolute ethanol.

The structure of compound **(II)** was confirmed by its reported melting point in addition to IR spectroscopy which revealed the presence of strong absorption bands at 3300 cm-1 attributed to (NH), and at 1665 cm-1, 1637cm-1 attributed to two (C=O) groups that appeared as broad band.

Compound **(III)** was first prepared in 1997[13] by the reaction of 2-[3-oxo-3,4-dihydroquinoxalin-2-yl]acetohydrazide **(II)** with hydrazine dihydrochloride in aqueous 10% hydrochloric acid. The structure of compound **(III)** was confirmed by its reported melting point and IR data which revealed strong absorption

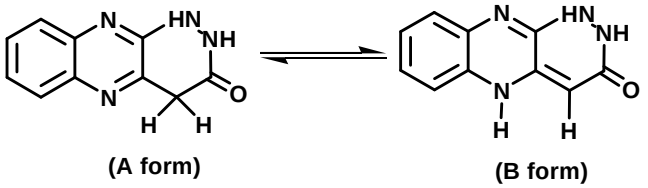


Scheme 2



Scheme 3

bands at 3310 cm-1 attributed to two (NH) groups and at 1664 cm-1 attributed to (C=O) group. The ^{1}HNMR spectrum of compound **(III)** in DMSO exhibited a tautomeric equilibrium between two forms (A and B forms). The C^4-methylene and C^4-vinylic proton signals were observed at δ 2.49 ppm (A form) and 6.42 ppm (B form) ppm, respectively as reported[13].



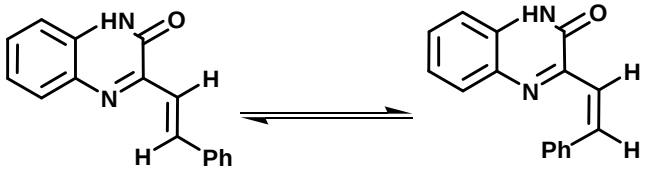
Several unsuccessful attempts were made to prepare compound **(IV)** through condensation of 1,4-dihydropyridazino[3,4-b]quinoxalin-3(2*H*)-one **(III)** with aromatic aldehydes in presence of sodium hydroxide or sodium acetate in all attempts the reported 3-(2-Arylethenyl)quinoxalin-2(1H)-ones V were formed[15].

The reaction of compound **(III)** with benzaldehyde

in aqueous sodium hydroxide or sodium acetate gave compound that has different microanalytical data from the expected compound. In addition, spectral data were different, thus IR spectrum of the product showed only one NH instead of two. Furthermore, ^{1}HNMR of the same compound showed the presence of only one exchangeable proton instead of two and ten aromatic protons instead of eleven. Repeating the reaction with other aldehydes (4-hydroxybenzaldehyde, 4-chlorobenzaldehyde, 4-dimethylaminobenzaldehyde, 4-nitrobenzaldehyde) gave similar data. These data directed our sight to the instability of compound **(III)** in basic medium. The pyridazine ring in this compound is nonaromatic and hence, it behaves like a lactam in basic medium.

To confirm this postulation, compound **(III)** was heated in aqueous sodium hydroxide and the product separated was 3-methylquinoxalin-2(1*H*)-one as shown by its reported melting point, microanalytical and spectral data[16].

The unexpected pathway of these reactions was proved through spectral data of the products (compounds **(Va-e)**) as follow:- IR spectra of compounds **(Va-e)** showed strong absorption bands at 3437-3177cm-1 (NH), 1662-1661 cm-1 (C=O), 1595-1590 cm-1 (C=C).The ^{1}HNMR spectrum of compounds **(Va)** showed the presence of peaks at ä 3.84 ppm (s, 1H, N1H, D2O exchangeable); 7.31-7.79 ppm (m, 10H, 9Ar-H and 1H of CH=CH-Ph); 8.04-8.09 ppm (d, 1H, CH=CH-Ph, J = 15 Hz high value of coupling constant of styryl protons indicated the presence of trans isomer at the double bond as illustrated below)[17].



trans isomer of compound (Va) cis isomer of compound (Va)

In scheme II, it was designed to incorporate arylazo moiety into quinoxaline nucleus. The usual coupling procedure is to add ice colded diazonium salt to the basic solution of active methylene containing compound. This treatment on compound **(I)** causes its hydrolysis and decarboxylation

Thus, acidic conditions was used to conduct the reaction namely acetic acid adopting the method of Kurasawa et al.[12,13] For synthesis of compound **(VIII)**, the usual coupling procedure on compound **(VII)** by adding ice coolded diazonium salt to the basic solution of active methylene containing compound was adopted. Previously, acidic conditions was used to conduct the reaction using acetic acid without buffer by Kurasawa et al.[18,19]

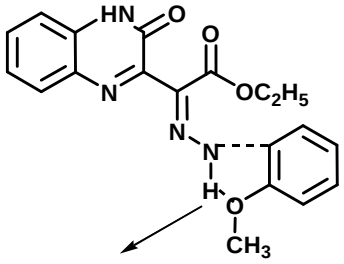
Later on, Widermanova et al at 1999 used acetic acid buffered with sodium acetate as a vehicle for the reaction.[20,21]

In this thesis the first method was utilized to carry out coupling reaction on compound **(VII)** as it was easier, consumed less chemicals and less time reaction and gave slight better yield than the other two methods.

The ^{1}HNMR spectrum of compounds **(VIa)** showed the presence of a tautomeric hydrazone imine form due to the presence of electron withdrawing ester group[18].

In addition to the presence of new peaks at δ 1.15-1.26 ppm (m, 3H, OCH_2CH_3), 3.30 ppm (s, 3H, Ph-CH_3), 4.09-4.19 ppm (m, 2H, OCH_2CH_3) attributed to syn, anti diastereomers of ethoxy group of the ester.

^{1}HNMR spectrum of compound **(VIc)** revealed the presence of two new peaks at δ at 12.56 ppm attributed to N1H and at 13.68 ppm attributed to N-NH proton D_2O exchangeable protons, which was



hydrogen bond

desheilded due to the intramolecular hydrogen bond with adjacent *o*- methoxy group as shown.

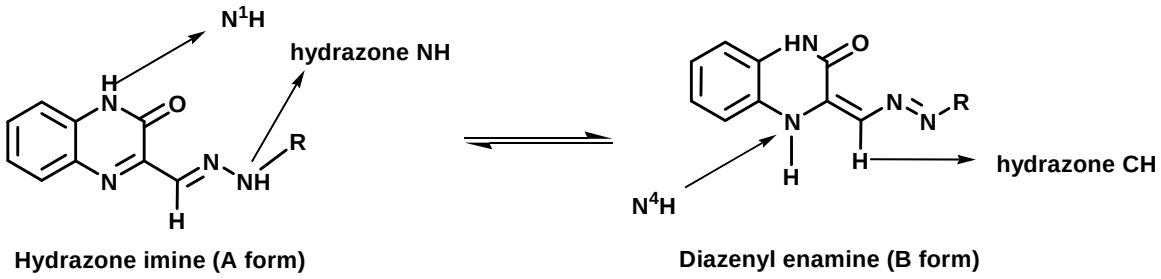
Compound **(VII)** was prepared by hydrolysis followed by decarboxylation of compound **(I)** by heating with aqueous potassium hydroxide followed by acidification with hydrochloric acid[14].

Compounds **(VIIIa-e)** were synthesized and their structures were confirmed by element and spectroscopic analysis.

IR spectra of compounds **(VIIIa,b,d,e)** showed

strong absorption band at 3465-3256 cm-1 (OH), compounds **(VIIIa-e)** showed strong absorption band at 3166-3134 cm-1 (NH).

^{1}HNMR spectrum of compounds **(VIIIa-e)**



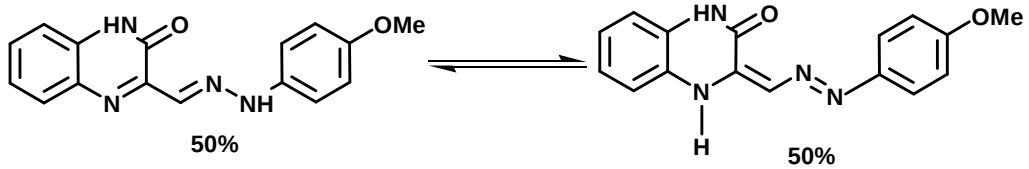
Hydrazone imine (A form) Diazenyl enamine (B form)

showed a tautomeric equilibrium between the hydrazone imine (A form) and diazenyl enamine (B form)[18].

^{1}HNMR spectrum of compounds **(VIIIa-e)** also revealed that the tautomeric ratio between the two forms depends on the position and nature of substituent Ar^2 that pronounced in ^{1}HNMR spectrum of compounds **(VIIIa)** and **(VIIIc)** .

^{1}HNMR spectrum of compounds **(VIIIa)** revealed the presence of eight aromatic protons at δ 6.83-7.68 ppm, doublet peak at 7.71-7.72 ppm attributed to hydrazone CH of both A, B forms, three D2O exchangeable protons at 10.19 ppm attributed to N4H proton of B form, 12.52 ppm attributed to N^{1}H proton and at 14.85 ppm attributed to OH proton, it was noted that hydrazone NH proton of A form was unobservable.

^{1}HNMR spectrum of compounds **(VIIIc)** revealed the presence of peak doublet peak at 7.90-7.95 ppm attributed to hydrazone CH of both A, B forms, two D_2O exchangeable protons at 7.68 ppm attributed to 0.5H of hydrazone NH proton of A form, 12.48 ppm attributed to N^{1}H proton and at 14.6 ppm attributed to 0.5H of hydrazone N^{4}H proton of B form, it was noted



that equilibrium ratio of A to B forms was 50% versus 50% as illustrated below.

^{1}HNMR spectrum of compounds **(VIIIf)** revealed the presence of doublet signal at 8.37-8.38 ppm attributed to hydrazone CH of both A & B forms, four D_2O exchangeable protons at 12.11 ppm indicated hydrazone NH, N^{1}H protons and at 12.54 ppm attributed to OH, COOH protons which appeared at the same signal due to intramolecular hydrogen bond formation that decreased resolution.

The common feature in the amines is that they are aminophenols. It is well known that phenols react with formaldehyde to form phenol-formaldehyde resins which are high melting and insoluble in all reagents. This effect is potentialized by the presence of the activating amino group.[22-26]

To confirm this postulation *p*-aminophenol is heated with formaldehyde in aqueous solution containing either hydrochloric acid, sodium hydroxide or none (neutral). In all cases the high melting solids which are insoluble in all solvents are formed.

Attempts to react compound **(VII)** with formaldehyde for 12 hours before adding aminophenol did not improve matters.

Hence, non phenolic amines are used in this reaction and compounds of the general formula shown are prepared.

The structure of the prepared compounds **(IXa-c)** was confirmed by elemental analysis and spectral data.

IR spectra of compounds **(IXa-c)** showed new absorption bands in the range of 3432-3361 cm-1 (indicated appearance of two NH bands), 2923-2842 cm-1 (CH aliphatic), in addition compounds **(IXc)** showed absorption band at 3483-3466 cm-1 attributed to OH group.

^{1}HNMR spectrum of compounds **(IXa)** revealed the presence of two peaks D_2O exchangeable protons at 3.45 ppm and 12.42 ppm attributed to CH_2NH, N^{1}H

protons respectively, CH_2NH proton was noted to be shielded due to adjacent *o*- methyl group with +I effect.

^{1}HNMR spectrum of compounds **(IXb)** revealed the presence of two peaks D_2O exchangeable protons at 4.21 ppm and 12.30 ppm attributed to CH_2NH, N^1H protons respectively, shielding effect of *p*- methyl group on CH_2NH proton was noted to be decreased.

^{1}HNMR spectrum of compounds **(IXc)** revealed the presence of three D_2O exchangeable protons at 4.32 ppm, 11.90 ppm and 12.36 ppm attributed to CH_2NH, N1H and COOH protons respectively.

Compound **(X)** was first prepared in 1948[27] by heating under reflux 2-oxo-3-methylquinoxaline with phosphoryl chloride. The solution was treated with ice-water then extracted with ether. Removal of ether gave red crystals of compound **(X)** which was used as starting material for further reactions.

The reaction of **(X)** with amines or salicylic acid was conducted in refluxing butanol containing catalytic amounts of potassium carbonate and potassium iodide.

^{1}HNMR spectra of compounds **(XIa-e)** indicated the presence of singlet peak at δ 2.70-2.78 ppm of CH_3 protons.

^{1}HNMR spectrum of compounds **(XIa)** revealed the presence of doublet peak at 6.47-6.50 ppm of one aromatic proton with *J* = 9 Hz. that affected by shielding effect of adjacent hydroxyl group with +M effect but in compounds **(XIc)**. revealed the presence of doublet peak at 9.34- 9.37 ppm of one aromatic proton with *J* = 9 Hz. that affected by deshielding effect of adjacent COOH group with -M effect.

Pharmacology

(A) Results

(a) Anti-inflammatory activity

Formalin induced paw edema was used for evaluating anti-inflammtory activity in this study.All the tested compounds demonstrated showed anti-inflammatory activity by reducing the paw thickness as compared with the control group that recorded in TABLE 1. The standard ketoprofen exhibited protection percent begins from 37.34 % at 30 min after formalin injection then increased gradually till reach 63.95 % at 3 h that decreased to 52.39 % at 48 h.

TABLE 1 : Evaluation of anti-inflammatory activity of the tested synthesized compounds (200mg/kg b.wt. P.O.) using formalin induced paw edema method.

group	Paw thickness (cm) (mean ± S..E.)						Inhibition percent (%)					
	30 min	1 h	2 h	3 h	24 h	48 h	30 min	1 h	2 h	3 h	24 h	48 h
Control 2% tween 80 p.o.	0.83 ± 0.004c	0.86 ± 0.005c	0.86 ± 0.005c	0.86 ± 0.005c	0.72 ± 0.004c	0.63 ± 0.006c	0	0	0	0	0	0
Ketoprofen 50mg/kgb.wt.	0.52 ± 0.012C	0.52 ± 0.012C	0.48 ± 0.014C	0.31 ± 0.007C	0.30 ± 0.008C	0.30 ± 0.007C	37.34	39.53	44.19	63.95	58.33	52.39
VIf	0.54 ± 0.012C	0.53 ± 0.017C	0.53 ± 0.017Ca	0.37 ± 0.017Cb	0.37 ± 0.016Cb	0.37 ± 0.018Cb	34.94	38.37	38.37	56.98	48.61	41.27
VId	0.58 ± 0.016Cb	0.58 ± 0.015Cb	0.58 ± 0.016Cc	0.42 ± 0.016Cc	0.40 ± 0.01Cc	0.38 ± 0.013Cc	30.12	32.56	32.56	51.63	44.44	39.68
XIb	0.60 ± 0.017Cb	0.62 ± 0.016Cc	0.53 ± 0.013Ca	0.48 ± 0.014Cc	0.42 ± 0.015Cc	0.36 ± o.o17CB	27.71	28.26	38.37	44.19	41.67	42.86
XIc	0.59 ± 0.018Cb	0.58 ± 0.015Cb	0.56 ± 0.013Cc	0.56 ± 0.014Cc	0.53 ± 0.015Cc	0.53 ± 0.015Cc	28.92	32.56	32.56	34.88	26.39	15.87
VIe	0.48 ± 0.017C	0.48 ± 0.022C	0.60 ± 0.022Cc	0.67 ± 0.023Cc	0.58 ± 0.020Cc	0.50 ± 0.021Cc	42.17	44.19	30.23	22.09	19.44	20.63
VIIId	0.44 ± 0.012Cb	0.50 ± 0.003C	0.65 ± 0.02Cc1	0.68 ± 0.019Cc	0.71± 0.018c	0.060 ± 0.012c	46.98	41.86	24.42	20.93	1.39	4.76
VIIIe	0.55 ± 0.029C	0.55 ± 0.029C	0.70 ± 0.005Cc	0.70 ± 0.015Cc	0.68 ± 0.017Ac	0.62 ± 0.004c	33.73	36.05	18.60	16.60	5.56	1.59
IXc	0.52 ± 0.017C	0.60 ± 0.013Cb	0.80 ± 0.015Bc	0.82 ± 0.012Bc	0.71 ± 0.014c	0.63 ± 0.006c	37.35	30.23	6.98	4.65	1.39	0

- A,B,C indicating significant differences as compared with control group (A p = 0.05, B p = 0.01 ,C p = 0.001)
- A,b,c indicating significant differences as compared with standard group (a p = 0.05, b p = 0.01 ,c p = 0.001)

Among these compounds tested, compound **(VId)**, **(VIf)**, **(XIb)** and **(XIe)** showed their highest paw thickness Inhibition percent (56.98, 51.63, 44.19 and 34.88, respectively) at 3 hours after formalin injection. However the compounds **(VIIId)**, **(IXc)** and **(XIe)** exhibited their highest inhibition percent at 30 mins. after formalin injection. Compounds **(VIf)** and **(VIIIe)** appeared their highest anti-inflammatory activity at 1h after induction of paw edema.

(b) Analgesic activity

Analgesic activity was evaluated by hot plate method and all tested compounds in a dose of 200 mg

/ kg b.wt. were orally demonstrated showed analgesic activity by increase in latency time when compared with the control group ($p = 0.05 – 0.001$) . The analgesic activity of the compound **(XIe)** was found to be superior with 216.90 % pain inhibition percent compared to other compounds. On the other hand, this followed by compound **(XIf)**, **(VIIIe)**, **(VIf)**, **(IXc)** and **(VId)** that showed pain inhibition percents ranged from 146.48 - 115.05 %. However lower percents ranged from 108.80 - 94.35 % were recorded for compounds **(VIe)**, **(XIc)** and **(XIb)**. The lowest activity was recorded for compound **(VIIId)** as it gave 67.38 % pain inhibition percent at 2 hours after oral administration.

(B) Discussion

(a) Anti-inflammatory activity

It is well known that acute inflammation in formalin induced paw edema model is biphasic, in the first phase there is a release of histamine, 5-HT and kinine, while there is a release of prostaglandins in the second phase[28].

Ketoprofen, the standard drug used showed anti-inflammatory activity all over the tested time beginning from 30 mins. till 48 hours, with maximum activity at 3 hours. Like other non stroidal anti-inflammatory drugs (NSAIDs) it acts by inhibiting both isoforms of cyclo-oxygenase enzyme responsible for the conversion of arachidonic acid into a variety of prostaglandins, thromboxanes and leukotreines[29].

So its activity is more pronounced on the second phase. All the tested compounds at 200 mg/kg P.O. possess anti-inflammatory activity compared with the control group. Compounds **(VIf)**, **(VId)**, **(XIb)** and **(XIe)** showed protective effect in formaline induced rat paw edema beginning from 30 mins. (1st phase) with highest activity at 3 hours (2nd phase). This is in accordance with their synthetic chemical structure, as compound **(VIf)** contains carboxylic group which responsible for the anti-inflammatory acrivity in some synthetic compounds As it competes carboxylic group of arachidonic acid so inhibits synthesis of prostaglandins[9,30].

Compound **(VId)** contain *p*-methoxy group which provides anti-inflammatory activity in previous synthetic compounds[9,30].

Compounds **(VIf)**, **(VId)**, **(XIb)** still having a sustained activity representing by high edema inhibition percent till 48 hours after induction of paw edema. In spite of compound **(XIb)** has a synthetic chemical structure near to paracetamol which is standard analgesic and antipyretic drug but not anti-inflammatory, it exhibites significant anti-inflammatory activity, this activity might be regarded to the main nucleus of this tested compound[31,32,37].

The anti-inflammatory activity of these compounds **(VIf)**, **(VId)**, **(XIb)** still sustained till 48 hours after edema induction. In contrary, a noticable decrease in the activity of compound **(XIe)** was recorded at 24 and 48 hours. As this compound has chemical structure resembling to salicylates which possess its highest anti-inflammatory action within 4 hrs after drug administration[33].

Tested compounds **(6)**, **(VIe)**, **(VIIId)**, **(VIIIe)**, **(IXc)**, and **(XIc)** showed significant anti-inflammatory activity compared to control group. All these compounds begin with their highest activity at 30 mins. following induction of paw edema with exception at 1hour for the compound **(VIIId)**. Then the activity of these compounds decreased stepwise till become non-significant after 24 and 48 hours, reached to zero inhibition percent (0%) at 48 hours for compound **(IXc)** and **(XIc)**.

Compounds **(XIf)** and **(VIIIe)** are analoge to salicylates while **(VIe)**, **(VIIId)**, **(IXc)**, and **(XIc)** are analoges to mephenamic acid, both of them are known to still active after 4 hours.[33,34]

Therefore lowered potency recorded after 1 hour for these tested compounds might be due to their rapid biotransformation in liver by acetylation of their carboxylic group. Furthur studies will be required to elucidate these points.

Among the compound tested, compound **(VIf)**, **(VId)** and **(XIb)** posses good anti-inflammatory activity with prolonged or persistence high significant effect till 48 hours. However compound **(VIe)** & **(VIIId)** demonstrate good but short activity sustained for 3 hours only compared to control group and standard group. Compound **(IXc)** & **(XIc)** possess good but very short anti-inflammatory activity as compared to both control and standard group.

(b) Analgesic activity

Concerning the analgesic activity, hot plate and tail

immersion methods are selective methods for screening centrally acting analgesics[34-36].

Our newly tested compounds are quinoxaline derivatives, thus, quinoxaline moiety exhibits analgesic activity via centrally mediated analgesic mechanism[37].

Hot plate method is used to evaluate the analgesic activity of these newly synthesized compounds.

The results demonstrated that all the tested compounds following oral administration (200mg/kg) possess marked analgesic profile represented by significantly prolonged latency time to heat stimulus in comparison to control group and ketoprofen (as standard).

Compound **(XIe)** was found to be the superior as analgesic one among the compounds tested, with pain inhibition percent of 216.90 % after 1 hour. Thus, it was thought that introduction of methyl group to the original nucleus in addition to presence of hydroxyl and carboxylic groups provide this highest recorded activity.

Previously (Insel[38] in 1991) suggested that substitution with methyl group in aspirin increases its anti-inflammatory and analgesic activities. Compounds **(VId)**, **(VIf)**, **(VIIIe)** and **(XIc)** have significant high analgesic activity in our study. From chemical structure point of view, compounds **(VIIIe)** and **(XIe)** resemble salicylates and both exert their highest activity after 1 hour.

From this it seemed that, presence of OH, COOH groups in position 3, 4 of NH group respectively in compound **(XIe)** improves analgesic activity On the other hand, compounds **(VIe)**, **(VIIId)**, and **(XIc)** and **(IXc)** have structure similarity close to mefenamic acid which is well known as potent analgesic drug. All these compounds except **(VIIId)** showed significant high analgesic activity, for compound **(VIIIe)** demonstrate its action very rapid after 30 mins. Then gradually decreased till 2 hours after dosing. However, compounds **(VIe)**, and **(IXc)** exhibit also high activity but more prolonged than **(VIIIe)**. According to the fact of structure activity relationship, it could be deduced that presence of hydroxyl group in compound **(VIIIe)** enhance its absorption and biotransformation resulting in rapid onset effect but with short dual activity due to rapid bioacetylation of hydroxyl group in liver.

Compound **(VIf)** exerts high analgesic activity with long lasting that begins very rapid after 30 mins. With maximum pain inhibition percent 134.86 % after 1 hour then decreased to 103.58 % after 2 hours. Compound **(VId)** exerts analgesic activity which appeared after 1 hour with increasing in its pain inhibition percent after 2 hours as compared to control group and that due to the presence of methoxy group which provides marked improvement of activity by formation of additional hydrogen bond with binding sites of COX enzyme.

Among all our tested compounds, compound **(VIIId)** shows the lowest analgesic activity as it only demonstrate significant activity after 2 hour with pain inhibition percent of 67.38 % and that may be due to its lack to the lipophilic moiety characterized compounds **(VIe)**, **(IXc)** and **(XIc)** that resulted in low absorption and low duration of action of compound **(VIIId)**

EXPERIMENTAL

Chemistry

Melting points were determined on a griffin apparatus and are uncorrected. IR spectra were determined as KBr discs on Shimadzu 435 spectrometer and values are represented in cm-1. ^{1}HNMR were carried out on Varian Gemini 200 or 300 MHz spectrometer, at the Microanalytical Center, Cairo University, Giza, Egypt, using TMS as internal standard and chemical shifts are recorded in ppm on ä scales. Mass spectra were run on Heweltt Packard 5988 spectrometer, at the Microanalytical Center, Cairo University, Giza, Egypt, and National Research Center, Giza, Egypt. Elemental analysis were carried out at the Microanalytical Center, Cairo University, Giza, Egypt. Progress of the reaction was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel MERCK 60 F 254 that was visualized by UV lamp.

Ethyl 3-oxo-3,4-dihydroquinoxalin-2-yl-acetate (I)[11]

A mixture of ethyl acetate (8.8g, 0.1mol) and diethyl oxalate (14.6g, 0.1mol) was added to a solution of sodium metal (2.3g, 0.1 atomic wt.) in absolute ethanol (25ml). The reaction mixture was stirred for 30 mins., then added to a solution of *o*-phenylene diamine (10.8g, 0.1mol), in mixture of hot ethanol (15ml) and acetic acid (7ml). The reaction mixture was heated on a steam bath for 10 mins., after cooling, the separated solid was filtered and crystallized from ethanol to give **(I)**. Yield:

20.50g (≈ 88.36%) m.p.: 205°c (as reported).

2-[3-Oxo-3,4-dihydroquinoxalin-2-yl]acetohydrazide (II)[21]

A mixture of compound **(I)** (9.28g, 0.04mol) and hydrazine hydrate 99% (21.08ml, 0.45mol) in absolute ethanol (200ml) was heated under reflux on a boiling water bath for 3hs. The reaction mixture was allowed to cool to room temperature. The separated solid was filtered and crystallized from ethanol to yield **(II)**. Yield: 8.54g (≈ 98%) m.p.: 264-266 °c (as reported)., I R (KBr γ cm-1) 3300 cm-1 (NH), 1665 cm-1 and 1637 cm-1 two (C=O) groups.

1,4-Dihydropyridazino[3,4-b]quinoxalin-3(2H)-one (III)[14]

A mixture of compound **(II)** (1.0g, 0.0045mol) and hydrazine dihydrochloride (4.83g, 0.046mol) in 10% hydrochloric acid (5ml) and water (50ml) was heated under reflux on a boiling water bath for 3hs. Evaporation of the solvent under reduced pressure, afforded yellow crystals which were collected and crystallized from ethanol to give **(III)**. Yield 87.9%; m.p.256-258 °C; IR (KBr): 3310 (2NH), and 1664(C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6): δ 2.49 (s, 1.5H, C4H of A form); 6.42 (s, 0.5H, C4H of B form); 7.22-7.74 (m, 4H, Ar-H); 11.58 (s, 1H, N1H, D2O exchangeable); 12.28 ppm (s, 1H, N2H, D2O exchangeable) and the N5H proton signal of B form was overlapped with other proton signals.

3-(2-phenylethenyl)quinoxalin-2(1H)-ones (V)

A mixture of compound **(III)** (1.0g, 0.005mol), sodium hydroxide (0.30g, 0.007mol) and the appropriate benzaldehyde (0.005mol) in ethanol (30ml), was heated under reflux for 4hs.then cooled. The obtained solid was filtered and crystallized from dioxane to give V. Yield 73%; m.p.252-254 °C; IR (KBr): 3299 (NH), 1661 (C=O) and 1590 (C=C) cm-1; ^{1}HNMR (300 MHz, DMSO-d6): δ 3.84 ppm (s, 1H, N1H, D2O exchangeable); 7.31-7.79 ppm (m, 10H, 9Ar-H and 1H of CH=CH-Ph) and 8.04-8.09 ppm (d, 1H, CH=CH-Ph with *J* =15 Hz.).; EIMS: *m/z* 248 (M^+) (6.82%);

General procedure for the synthesis of VIa-f

A solution of sodium nitrite (6.9g, 0.1mol) in water (50ml) was added to the appropriate aromatic amine (0.1mol) in 10% hydrochloric acid (30ml) and acetic acid (70ml) in an ice bath to give a clear solution, which was added to a suspension of compound **(I)** (11.6g, 0.05mol) in acetic acid (50ml) with stirring for 10 min. in an ice bath. Stirring was continued for additional 10 mins., the mixture was heated in a boiling water bath for 1hr. then the reaction mixture was cooled to room temperature and the precipitated crystals were filtered off and crystallized from the appropriate solvent to afford **(VIa-f)**.

Syn and anti ethyl 2-(2-methylphenylhydrazin-1-ylidene)-2-(3-oxo-3,4-dihydroquinoxalin-2-yl)acetates(VIa)

Crystallization solvent is ethanol, Yield 67%; m.p.190-192°C; IR(KBr): 3434(NH), 2918 (CH, aliph.) and 1650 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6): δ 1.15-1.26 ppm (m, 3H, CH2CH3); 3.30 ppm (s, 3H, Ph-CH3); 4.09-4.19 ppm (m, 2H, CH2CH3); 6.99-7.39 ppm (m, 8H, Ar-H); 11.04 ppm (s, 1H, N-NH, D2O exchangeable) and 11.70 ppm (s, 1H, N1H, D2O exchangeable). EIMS: *m/z* 250 (M^+) (81.28%); 351(21.84%) $(M^{+1})^{+\cdot+}$; 105(100%). Anal. Calcd for $C_{19}H_{18}N_4O_3$: C, 65.13; H, 5.18; N, 15.99. Found; C, 65.14; H, 5.14; N, 16.22%.

Syn and anti ethyl 2-(4-methylphenylhydrazin-1-ylidene)-2-(3-oxo-3,4-dihydroquinoxalin-2-yl)acetates(VIb)

Crystallization solvent is ethanol, Yield 82%; m.p.183-185 °C; IR(KBr):3425(NH), 2920 (CH, aliph.) and 1675 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6): δ 1.17-1.24 ppm (m, 3H, CH2CH3); 2.25 ppm (s, 3H, Ph-CH3); 4.17-4.20 ppm (m, 2H, CH2CH3); 7.13-7.86 ppm (m, 8H, Ar-H); 11.15 ppm (s, 1H, N-NH, D2O exchangeable) and 12.60 ppm (s, 1H, N1H, D2O exchangeable).Anal. Calcd for $C_{19}H_{18}N_4O_3$: C, 65.13; H, 5.18; N, 15.99. Found; C, 65.14; H, 5.14; N, 16.13%.

Syn and anti ethyl 2-(2-methoxyphenylhydrazin-1-ylidene)-2-(3-oxo-3,4-dihydroquinoxalin-2-yl)acetates(VIc)

Crystallization solvent is butanol, Yield 55%; m.p.253-255 °C; IR (KBr): 3437(NH), 2835 (CH, aliph.), 1729 (C=O)and 1666 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6): δ 1.15-1.27 ppm (m, 3H, CH2CH3); 3.49 ppm (s, 3H, OCH3); 4.19-4.26 ppm

(m, 2H, CH2CH3); 7.00-7.79 ppm (m, 8H, Ar-H); 12.56 ppm (s, 1H, N1H, D2O exchangeable) and 13.68 ppm (s, 1H, N-NH, D2O exchangeable);. EIMS: *m/z* 366 (51.94%) $(M)^{+\cdot}$; 121(100%).Anal. Calcd for $C_{19}H_{18}N_4O_4$: C, 62.29; H, 4.95; N, 15.29. Found; C, 62.19; H, 5.26; N, 15 %.

Syn and anti ethyl 2-(4-methoxyphenylhydrazin-1-ylidene)-2-(3-oxo-3,4-dihydroquinoxalin-2-yl)acetates(VId)

Crystallization solvent is butanol, Yield 45%; m.p.240-242 °C; IR (KBr): 3433(NH), 2846 (CH, aliph.) 1729 (C=O) and 1664 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6): δ 1.18-1.23 ppm (m, 3H, CH2CH3); 3.72 ppm (s, 3H, OCH3); 4.17-4.22 ppm (m, 2H, CH2CH3); 6.90-7.89 ppm (m, 8H, Ar-H); 11.20 ppm (s, 1H, N-NH, D2O exchangeable) and 12.56 ppm (s, 1H, N1H, D2O exchangeable). Anal. Calcd for C19H18N4O4: C, 62.29; H, 4.95; N, 15.29. Found; C, 62.36; H, 5.30; N, 15.05 %.

Syn and anti 2-{N1-[Ethoxycarbonyl (3-oxo-3,4-dihydroquinoxalim-2-yl)methylene] hydrazino} benzoic acid (VIe)

Crystallization solvent is ethanol, Yield 67%; m.p.185-187 °C; IR (KBr): 3460(OH), 3320 (NH.), 2923 (CH aliph.) and 1656 (br. C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6): δ 1.14-1.27 ppm (m, 3H, CH2CH3); 4.06-4.23 ppm (m, 2H, CH2CH3); 6.99-8.20 ppm (m, 8H, Ar-H); 11.03 ppm (s, 1H, N-NH, D2O exchangeable); 11.75 ppm (s, 1H, N1H, D2O exchangeable) and 12.54 ppm (s, 1H, COOH, D2O exchangeable). EIMS: *m/z* 381(12.35%) $(M^{+1})^{+\cdot}$; 43(100%). Anal. Calcd for C19H16N4O5: C, 60; H, 4.24; N, 14.73. Found; C, 60.12; H, 4.21; N, 14.79 %.

Syn and anti 4-{N1-[Ethoxycarbonyl(3-oxo-3,4-dihydroquinoxalim-2-yl) methylene] hydrazino} benzoic acid (VIf)

Crystallization solvent is ethanol, Yield 72%; m.p.193-195 °C; IR (KBr): 3460(OH), 3342 (NH.), 2923 (CH aliph.), 1673 (C=O) and 1655 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6): δ 1.20-1.25 ppm (m, 3H, CH2CH3); 4.18-4.25 ppm (m, 2H, CH2CH3); 7.27-7.91 ppm (m, 8H, Ar-H); 11.19 ppm (s, 1H, N-NH, D2O exchangeable); 12.00 ppm (s, 1H, N1H, D2O exchangeable) and 12.80 ppm (s, 1H, COOH, D2O exchangeable). Anal. Calcd for C19H16N4O5: C, 60; H, 4.24; N, 14.73. Found; C, 60.30; H, 4.21; N, 14.75 %.

3-Methylquinoxalin-2(1H)-one (VII)[11]

A mixture of compound **(I)** (16.24g, 0.07mol) was dissolved in water (30ml) containing potassium hydroxide (3.92g, 0.07mol), then boiled for 30 mins., concentrated hydrochloric acid was added drop wise until the solution became acidic to litmus paper. The separated solid was filtered and crystallized from ethanol to yield compound **(II)**. Yield: 8.06g (≈ 72%) (as reported). m.p. : 250-252°c.

General procedure for the synthesis of VIIIa-f

A solution of $NaNO_2$ (6.9g, 0.1mol) in ice-cold water (40ml) was added portionwise with stirring to a solution of the corresponding aromatic amine (0.1mol) in a mixture of conc. hydrochloric acid (3.0ml) and water (10-30ml). The resulted solution was stirred in an ice-bath for 30 min. And left to stand for 30-60 mins. then added portionwise during 10 mins. to a stirred mixture of compound **(VII)** (16.0g, 0.1mol) in water (10ml) and acetic acid (30ml). The separated crystals were filtered and crystallized from the appropriate solvent to give **(VIIIa-f)**

3-[(2-Hydroxyphenylhydrazin-1-ylidene) methyl] quinpxalin-2(1H)-one (VIIIa)

Crystallization solvent is propanol, Yield 63%; m.p.244-246 °C; IR (KBr): 3256(OH), 3162 (NH.), and 1673 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6): δ 6.83-7.68 ppm (m, 8H, Ar-H); 7.71-7.72 ppm (d, 1H, CH of both hydrazone imine and diazinyl enamine forms); 10.19 ppm (s, 1H, N4H of diazenyl enamine form, D2O exchangeable); 12.52 ppm (s, 1H, N1H, D2O exchangeable) and 14.85 ppm (s, 1H, OH, D_2O exchangeable) and hydrazone NH proton was unobservable . EIMS: *m/z* 280(9.92) $(M^{+\cdot})^{+\cdot}$; 63(100%). Anal. Calcd for $C_{15}H_{12}N_4O_2$: C, 64.28; H, 4.32; N, 19.99. Found; C, 64.20; H, 4.27; N, 19.98 %.

3-[(4-Hydroxyphenylhydrazin-1-ylidene)methyl]quinpxalin-2(1H)-one (VIIIb)

Crystallization solvent is propanol, Yield 81%; m.p.247-249 °C; IR (KBr): 3395(OH), 3165 (NH.), and 1655 (C=O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d6): δ 6.83-7.97 ppm (m, 8H, Ar-H); 8.35-8.38 ppm (d, 1H, CH of both hydrazone imine and diazenyl enam-

ine forms); 12.29 ppm (s, 1H, N4H of diazenyl enamine form, D2O exchangeable) 12.59 ppm (s, 1H, N1H, D2O exchangeable); 15.21 ppm (s, 1H, OH, D2O exchangeable) and hydrazone NH proton was unobservable. Anal. Calcd for $C_{15}H_{12}N_4O_2$: C, 64.28; H, 4.32; N, 19.99. Found; C, 64.30; H, 4.22; N, 20.02 %.

3-[(4-Methoxyphenylhydrazin-1-ylidene)methyl]quinpxalin-2(1H)-one (VIIIc)

Crystallization solvent is ethanol, Yield 76%; m.p.272-274 °C; IR (KBr): 3164 (NH.), and 1673 (C=O) cm^{-1}. ^{1}HNMR(DMSO-d6) for VIIIc : δ 3.74 (s, 3H, OCH_3); 6.94-7.50 (m, 8H, Ar-H); 7.68 (s, 0.5H, hydrazone NH, D2O exchangeable); 7.90- 7.95 (d, 1H, CH of both hydrazone imine and diazenyl enamine forms); 12.48 (s, 1H, N1H, D_2O exchangeable) and 14.60 ppm (s, 0.5H, N4H of diazenyl enamine form, D_2O exchangable). EIMS: *m/z* 294(80.7) $(M^{+\cdot})^{+\cdot+}$; 122(100%). Anal. Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found; C, 65.39; H, 4.79; N, 19.24 %.

2-[N/(3-Oxo-3,4-dihydroquinoxalin-2-ylmethylene)hydrazine]benzoic acid (VIIId)

Crystallization solvent is methanol, Yield 40%; m.p.290-292 °C; IR (KBr): 3410(OH), 3146 (NH.), and 1667 (2C=O) cm^{-1}. ^{1}HNMR(DMSO-d6) for VIIId : δ 7.00-7.97 ppm (m, 8H, Ar-H); 8.36-8.39 ppm (d, 1H, CH of both hydrazone imine and diazenyl enamine forms); 11.91 ppm (s, 0.5H, N4H of diazenyl enamine form, D_2O exchangeable); 12.30 ppm (s, 0.5H, hydrazone NH, D_2O exchangeable); 12.58 ppm (s, 1H, N1H, D_2O exchangeable) and 15.24 ppm (s, 1H, COOH, D2O exchangeable). EIMS: *m/z* 308(8.5) $(M^{+\cdot})^{+\cdot+}$; 131 (100%). Anal. Calcd for $C_{16}H_{12}N_4O_3$: C, 62.33; H, 3.92; N, 18.17. Found; C, 62.33; H, 3.70; N, 18.19 %.

2-Hydroxy-5-[N/(3-Oxo-3,4-dihydroquinoxalin-2-ylmethylene) hydrazine] benzoic acid (VIIIe)

Crystallization solvent is ethanol, Yield 70%; m.p.335-337 °C; IR (KBr): 3449(OH), 3166 (NH.), and 1668 (2C=O) cm^{-1}. ^{1}HNMR(DMSO-d6) for VIIId : δ 7.29-7.79 ppm (m, 7H, Ar-H); 8.37-8.38 ppm (d, 1H, CH of both hydrazone imine and diazenyl enamine forms); 12.11 ppm (s, 2H, hydrazone NH and N1H, D2O exchangeable); 12.54 ppm (s, 2H, OH and COOH, D2O exchangeable) and N4H proton of diazenyl enamine form was unobservable. Anal. Calcd for $C_{16}H_{12}N_4O_4$: C, 59.26; H, 3.73; N, 17.28. Found; C, 59.22; H, 3.80; N, 17.28 %.

General procedure for the synthesis of (IXa-c)

A mixture of compound **(VII)** (1.60g, 0.01mol), (37- 40%) formaldehyde solution (0.97ml, 0.012mol) and the appropriate amine (0.012mol) was heated under reflux for 10hs. then cooled. The separated crystals were collected by filteration and crystallized from the appropriate solvent to afford **(IXa-c)**.

3-(2-Methylphenylaminoethyl)quinoxaline-2(1H)-one (IXa)

Crystallization solvent is ethanol, Yield 65%; m.p.215-217 °C; IR (KBr): 3431 (NH.), 2842(CH, aliph.) and 1657 (C=O) cm^{-1}. ^{1}HNMR(DMSO-d6) for IXa: δ 1.03-1.08 (t, 2H, CH_2CH_2N-); 2.52 (s, 3H, Ph-CH_3); 3.45 (s, 1H, CH_2NH, D_2O exchangeable); 3.49-3.53 (t, 2H, CH_2CH_2N-); 7.17-7.79 (m, 8H, Ar-H) and 12.42 ppm (s, 1H, N1H, D_2O exchangeable). EIMS: *m/z* 279(8.18) $(M^{+\cdot})^{+\cdot+}$; 132 (100%). Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found; C, 73.25; H,6.39 ; N, 15.38 %.

3-(4-Methylphenylaminoethyl)quinoxaline-2(1H)-one (IXb)

Crystallization solvent is butanol, Yield 47%; m.p.223-225 °C; IR (KBr): 3423 (NH.), 2847(CH, aliph.) and 1658 (C=O) cm^{-1}. ^{1}HNMR(DMSO-d6) for IXb: δ 1.03-1.08 (t, 2H, CH_2CH_2N-); 2.53 (s, 3H, Ph-CH_3); 3.38-3.45 (t, 2H, CH_2CH_2N-); 4.21 (s, 1H, CH_2NH, D_2O exchangeable); 7.07-7.70 (m, 8H, Ar-H) and 12.30 ppm (s, 1H, N1H, D_2O exchangeable). Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found; C, 72.97; H,6.22 ; N, 15.11 %.

2-[2-(3-Oxo-3,4-dihydroquinoxalin-2-yl)-ethylamino]benzoic acid (IXc)

Crystallization solvent is ethanol, Yield 44%; m.p.275-277 °C; IR (KBr): 3483 (OH.),3361(NH), 2905(CH, aliph.) and 1667 (C=O) cm^{-1}. ^{1}HNMR(DMSO-d6) for IXc: δ 1.02-1.08 ppm (t, 2H, CH2CH2N-); 3.38-3.53 ppm (t, 2H, CH2CH2N-); 4.32 ppm (s, 1H, CH2NH, D2O exchangeable); 6.73-7.47 ppm (m, 8H, Ar-H); 11.9 ppm (s, 1H, N1H, D2O exchangeable) and 12.36 ppm (s, 1H, COOH, D2O exchangeable). EIMS: *m/z* 310 (1) $(M^{+\cdot})^{+\cdot+}$; 63 (100%). Anal. Calcd for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N,

13.58. Found; C, 66.09; H,5.12 ; N, 13.24 %.

2-Chloro-3-methylquinoxaline(X)[27]

Compound **(VII)** (8.00g, 0.05mol) was heated under reflux with freshly distilled phosphorous oxychloride (25ml) for 30 mins. The solution was concentrated under reduced pressure and the residue was treated with ice cold water. The produced solution was extracted with ether (3 x 25ml). The ether extract was dried over anhydrous Na_2SO_4 (20g.). The extract was filtered and ether was evaporated to give compound **(III)** which was solidified to red mass which was crystallized from ethanol to give red crystals. Yield: 6.20 g (≈ 69.66%), Mp : 84-86°c (as reported).

General procedure for the synthesis of XIa-e

A mixture of compound **(X)** (1.78g, 0.01mol), the appropriate amine (0.012mol), sodium iodide (0.13g, 0.001mol) and anhydrous potassium carbonate (0.138g, 0.001mol) in butanol (20ml) was heated under reflux for 24 hs. The reaction mixture was filtered while hot, concentrated and cooled. The separated product was crystallized from the appropriate solvent to afford **(XIa-e)**.

2-(3-Methylquinoxalin-2-ylamino)phenol (XIa)

Crystallization solvent is butanol, Yield 70%; m.p.150-152 °C; IR (KBr): 3427 (OH.),3176(NH) and 2957(CH, aliph.) cm^{-1}. ^{1}HNMR(DMSO-d6) for XIa δ 2.71 ppm (s, 3H, -CH3); 6.47-6.50 ppm (d, 1H, Ar-H with *J* = 9 Hz.); 7.09-7.78 ppm (m, 7H, Ar-H); 8.48 ppm (s, 1H, -NH, D2O exchangeable) and 9.37 ppm (s, 1H, -OH, D2O exchangeable). EIMS: *m/z* 251 (17.24) $(M^{+\cdot})^{+\cdot}$; 212 (100%). Anal. Calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found; C, 71.60; H, 5.16 ; N, 16.79 %.

4-(3-Methylquinoxalin-2-ylamino)phenol (XIb)

Crystallization solvent is butanol, Yield 62%; m.p.155-157 °C; IR (KBr): 3696 (OH.), 3127(NH) and 2856(CH, aliph.) cm^{-1}. ^{1}HNMR(DMSO-d6) for XIb δ 2.51 ppm (s, 3H, -CH3); 7.03-7.70 ppm (m, 8H, Ar-H); 11.87 ppm (s, 1H, NH, D2O exchangeable) and 12.26 ppm (s, 1H, -OH, D2O exchangeable). Anal. Calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found; C, 71.70; H, 5.16 ; N, 16.72 %.

2-(3-Methylquinoxalin-2-ylamino)benzoic acid (XIc)

Crystallization solvent is ethanol, Yield 50%; m.p.295-297 °C; IR (KBr): 3450 (OH.), 3106(NH), 2960(CH, aliph.) and 1666(C=O) cm^{-1}. ^{1}HNMR (DMSO-d6) for XIc: δ 2.78 ppm (s, 3H, -CH3); 7.06-8.09 ppm (m, 7H, Ar-H); 9.34- 9.37 ppm (d, 1H, Ar-H with *J* = 9 Hz.); 12.05 ppm (s, 1H, -NH-, D2O exchangeable) and 12.52 ppm (s, 1H, -COOH, D2O exchangeable). EIMS: *m/z* 279 (11.65) $(M^{+\cdot})^{+\cdot}$; 132 (100%). Anal. Calcd for $C_{16}H_{13}N_3O_2$: C, 68.81; H, 4.69; N, 15.04. Found; C, 68.80; H, 4.63 ; N, 15.03 %.

4-(3-Methylquinoxalin-2-yloxy)benzoic acid (XId)

Crystallization solvent is ethanol, Yield 47%; m.p.180-182 °C; IR (KBr): 3411 (OH.), 2894(CH, aliph.) and 1665(C=O) cm-1. ^{1}HNMR(DMSO-d6) for XId: δ 2.75 ppm (s, 3H, -CH3); 7.09-8.10 ppm (m, 7H, Ar-H); 9.37-9.40 ppm (d, 1H, Ar-H with J = 9 Hz.) and 11.55 ppm (s, 1H, -COOH, D_2O exchangeable). Anal. Calcd for $C_{16}H_{12}N_2O_3$: C, 68.57; H, 4.32; N, 9.99. Found; C, 68.57; H, 4.28 ; N, 9.80 %.

2-Hydroxy-4-(3-methylquinoxalin-2-ylamino)benzoic acid (XIe)

Crystallization solvent is ethanol, Yield 43%; m.p.225-227 °C; IR (KBr): 3412 (OH.), 3161 (NH), 2893(CH, aliph.) and 1666(C=O) cm-1. ^{1}HNMR (DMSO-d6) for XIe: δ 2.70 ppm (s, 3H, -CH3); 6.46-6.48 ppm (d, 1H, Ar-H with J = 6 Hz.); 7.12-7.77 ppm (m, 7H, 6Ar-H and D2O exchangeable OH proton); 8.44 ppm (s, 1H, -NH, D2O exchangeable) and 9.31 ppm (s, 1H, -COOH D2O exchangeable). EIMS: *m/z* 294 (1.23) $(M^{+\cdot}\text{-}1)^{+\cdot}$; 63 (100%). Anal. Calcd for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found; C, 65.00; H, 4.40; N, 14.46 %.

Anti-inflammatory and analgesic screening

Material and methods

Animals

Swiss strain male mice (4 weeks age), weighing between 25 and 30gm and wister albino rats of both sex with average body weight of 150gm were used in the experiments. The animals were purchased from Ophthalmic Research Institute, Giza, Egypt., were kept in polyethylene boxes (n = 6), in a controlled environment, constant temperature (24 ± 2 °C) with a 12 h light-dark cycle and relative humidity of 40–70%. They were kept without food for 24 hours before the experiment and water was *ad libitum*. This study was car-

ried out in accordance with current guidelines for the veterinary care of laboratory animals[14], and were approved by institutional animal ethics committee

Acute toxicity assessment

Acute toxicity of all synthesized compounds were studied on male albino mice according to the method of (Karber 1941) four groups of 5 mice each were used for each compound. Tween 80 suspension (2% v/v) of the tested compounds were administered orally in a graduated increased doses (500, 1000, 1500and 2000mg/kg.b.wt.). They were observed for 6 hours to detect any toxic symptoms, mortalities were recorded in each group after 24h from dosing.

All compounds were devoid of any toxicity in rats when given in doses up to 2000 mg/kg by oral route. Hence, in our study 200 mg/kg dose of each compound was suspended in 2 % tween 80 (v/v) and used for the study.

Anti-inflammatory activity

The anti-inflammatory activity was evaluated by formalin induced paw edema method according to Turner (1965)[28]. Wister strain albino rats were divided into twelve groups each of five animals . The paw thickness (0 hour) of each rat was measured, in millimeters by Vernier calliper. The first group was orally administered tween 80 suspension (2% v/v, 2ml/rat) and kept as control group.

The second one was orally given ketoprofen suspension in a dose of 50 mg/kg.b.wt. (standard group). The tested compounds were administered orally in the form of Tween 80 suspensions (2% v/v) 200mg/kg.b.wt. to the rest 10 groups. After one hour of the administration of the tested compound paw edema was induced in each rat by injecting 0.1ml formalin 2.5% subcutaneously into the right hind paw of each rat. The paw thickness of each rat was measured using caliber after 30 min, 1, 2, 3, 24 and 48h after formalin injection. The edema thickness (mm) was calculated by subtracting the zero-hour reading from each time reading. From the mean edema thickness, the percentage inhibition of the edema was calculated between the treated and control groups.

Percent (%) inhibition = $V_c - V_t \times 100 / V_c$

Where, V_C and V_t represent the average paw thickness in the control and treated groups, respectively.

Analgesic activity

Hot plate method

The hot plate test described by Eddy and Leimback (1953) was used .The animals were divided into ten groups of 5 animals each. Group I served as control, was administered tween 80 (2% v/v, 0.2ml/mice), Group II served as standard and was administered ketoprofen oral suspension in a dose of 50 mg/kg. The rest groups were treated orally with the tested compounds in the form of tween 80 suspension (2% v/v) 200 mg/kg.b.wt .The animals were individually placed

TABLE 2 : Evaluation of analgesic activity of the tested synthesized compounds (200 mg/kg. b.wt., P.O.) using hot plate method.

group	Latency time (second) (mean ± S.E.)			Pain protection percent		
	30 min	1 h	2 h	30min	1h	2h
Control (2% tween 80 p.o.)	2.83 ± 0.17c	2.84 ± 0.16c	2.79 ± 0.16 c	0	0	0
Ketoprofen (50 mg/kg) (p.o.)	5.87 ± 0.53C	7.24 ± 0.59C	9.24 ± 0.86C	107.42	154.92	231.18
VIf	6.0 ± 0.23C	6.67 ± 0.33C	5.68 ± 0.42Cb	112.01	134.86	103.58
VId	4.0 ± 0.52a	5.33 ± 0.35Ca	6.0 ± 0.56Ca	41.34	87.68	115.05
XIb	5.5 ± 0.48C	4.67 ± 0.31Ca	4.33 ± 0.52 Ac	94.35	64.44	55.19
XIc	6.33 ± 0.58C	9.0 ± 0.57C	5.0 ± 0.58 Bb	123.67	216.90	79.21
Vie	5.33 ± 0.42C	5.93 ± 0.65C	5.67 ± 0.45Cb	88.34	108.80	103.23
VIIId	3.17 ± 0.44b	3.33 ± 0.35c	4.67 ± 0.43Cb	12.014	17.25	67.38
VIIIe	6.67 ± 0.74C	5.73 ± 0.36C	4.33 ± 0.38Bc	135.69	101.76	55.197
IXc	5.67 ± 0.67B	6.32 ± 0.34C	5.33 ± 0.33Cb	100.35	122.54	91.04
XIc	6.0 ± 0.58C	7.0 ± 0.58C	5.0 ± 0.53Cb	100.71	87.68	87.09

- A, B, C indicating significant differences as compared with control group (A p = 0.05, B p = 0.01, C p = 0.001)

- A, b, c indicating significant differences as compared with standard group (a p = 0.05, b p = 0.01, c p = 0.001)

on the hot plate, maintained at 55°C, one hour after their respective treatments. The time taken by the animals to lick the hind paw or jump out of the place was taken as the reaction time, whichever appeared first, and was measured at 0, 30, 60 and 120 *mins*. a cut-off period of 15 *sec* was considered as maximal latency to avoid injury to the paws.

The pain inhibition percentage (PIP) was calculated according to the following formula:

Pain inhibition percentage (PIP) = $(T_t - T_c) / T_c \times 100$

T_t is drug latency time and T_c is control latency time.

Statistical analysis

The results were analyzed by One Way Analysis of Variance followed by the Student T-test. For the statistical analysis Sigma Stat (SPSS Inc, USA) was used.

REFERENCES

[1] O.I.El-Sabbagh, M.E.El-Sadek, S.M.Lashine, S.M.El-Nabtity; J.Med.Chem.Research, **18(9)**, 782-797 **(2009)**.
[2] N.Umarani, K.Ilango, K.S.Narenndra; J.Der Pharma.Chemica., **2(1)**, 159-167 **(2010)**.
[3] N.A.Punchard, S.M.Greenfield, R.P.H.Thompson; J.Mediator of Inflammation, **1**, 151-165 **(1992)**.
[4] O.O.Fadeyi, C.A.Obafemi, C.O.Adewunmi, E.O.Iwalewa; African Journal of Biotechnology, **3(8)**, 426-431 **(2004)**.
[5] A.M.Abdel-Alim, A.A.Elshorbagi, S.G.Abdel-Moty, H.H.Abdel-Allah; J.Arch.Pharm.Research, **6**, 637-647 **(2005)**.
[6] S.Keinanen, S.Simila, K.Kouvalinen; European Journal of Clinical Pharmacology, **13(5)**, 331-334 **(1978)**.
[7] A.H.Abadi, G.H.Hegazy, A.A.El-Zaher; J.Bioorganic and Medicinal Chemistry, **13**, 2759-2765 **(2005)**.
[8] M.Srivastav, M.Salahuddin, S.M.Shantakumar; European Journal of Chemistry, **6(4)**, 1055-1062 **(2009)**.
[9] S.K.Sahu, M.Banerjee, A.Samantray, C.Behera, M.A.Azam; Tropical Journal of Pharmaceutical Research, **7(2)**, 961-968 **(2008)**.
[10] K.Kaymakcioglu, A.Toklu, H.Z.Kabasakal; J.Archiv der Pharmazie, **339(5)**, 267-272 **(2006)**.
[11] D.W.Rangnekar, V.R.Kanethar, G.S.Shankarling, J.V.Malanker, C.R.Shanbahag; J.Heterocyclic Chem., **36**, 1213 **(1999)**.
[12] Y.Kurasawa, M.Muramatsu, K.Yamazaki, Y.Okamoto, A.Takada; J.Heterocyclic Chem., **31(11)**, 3879-3901 **(1983)**.
[13] Y.Kurasawa, M.Muramatsu, K.Yamazaki, Y.Okamoto, A.Takada; J.Heterocyclic Chem., **34(1)**, 39-42 **(1997)**.
[14] G.Westphal, H.Wasicki, F.Zielinski, F.G.Weber, M.Tonew, E.Tonew; Pharmazie, **32(H.10)**, 570 **(1977)**.
[15] M.Z.A.Bader, G.M.Elnaggar, H.A.H.Elsherief, S.A.Mahgoub; J.Chemical Society of Japan, **57**, 1633-1657 **(1984)**.
[16] Y.Hong, H.Kim, Y.Park, S.Kim; J.Bull Korean Chem. Soc., **21(1)**, 133-136 **(2000)**.
[17] M.M.Ali, M.M.F.Ismail, M.S.A.El-Gaby, M.A.Zahran, Y.A.Ammar; Molecules, **5**, 864 **(2000)**.
[18] Y.Kurasawa, M.Muramatsu, K.Yamazaki, Y.Okamoto, A.Takada; J.Heterocyclic Chem., **26**, 1129 **(1989)**.
[19] Y.Kurasawa, M.Muramatsu, K.Yamazaki, Y.Okamoto, A.Takada; J.Heterocyclic Chem., **31**, 527 **(1993)**.
[20] I.Wendermannova, J.Magdonova, J.Slouka; J.Chemica, **38**, 83-90 **(1999)**.
[21] I.Wendermannova, J.Magdonova, J.Slouka; J.Arkivok, **15**, 65-74 **(2003)**.
[22] K.K.Johnson, L.E.Jones; J.Ceramic Engineering and Material Science, **1**, 478 **(2000)**.
[23] D.P.K.Roy, H.Basu, S.V.Eswaran; J.Resonance, **1**, 59-66 **(2002)**.
[24] Y.K.Lee, D.Kim, T.S.Hwang, M.Refailovich, J.Sokolov; Journal of Applied Polymer Science, **89**, 2589-2596 **(2003)**.
[25] J.Paju, T.Pehk, P.Christjanson; J.Polymer Science, **58(1)**, 45-52 **(2009)**.
[26] D.Jiang, A.Duin, W.A.Goddard, S.Dai; Journal Physical Chemistry, **113**, 6891-6894 **(2009)**.
[27] G.T.Newbold, F.S.Spring; Journal Ammerican Chemical Society, **1**, 519-523 **(1948)**.
[28] R.A.Turner, P.Hebban; Analgesic Screening Methods in Pharmacology. Academic Press, New York, 100 **(1965)**.
[29] S.David, A.Kaye, A.Jason, S.J.Nichollas; J.Amm. Coll.Cardiol., **48**, 715-720 **(2006)**.
[30] B.Glopal, N.Raghunanadan, J.V.Rao, A.Venkatesham, M.Sarangapani; J.Indian Drugs, **6**, 42 **(2005)**.
[31] S.Ganapathy, H.Razoi, C.Moote, I.Yee, S.Geverdys; Candian J.Anesth., **43**, 1030-1039 **(1996)**.

[32] J.Sandeep, A.Deep, P.C.Shamara; J.Acta Poloniae Pharmaceutica, **67**, 63-67 **(2010)**.
[33] A.Khalaj, M.Abdollahi, N.Adibour, S.Rasoulamani; Indian Journal of Pharmacology, **34**, 184-188 **(2002)**.
[34] E.A.Siegmund, R.A.Cadmus; J.Proc.Soc.Biol. Med., **95**, 729 **(1957)**.
[35] M.F.Monte, G.Santos, G.M.Cunha; Journal Pharmacol Research, **49**, 239-243 **(2004)**.
[36] M.Hossain, S.M.Raquibal, R.Akter, N.Islam, J.Rashid; Stamford Journal of Pharmaceutical Sciences, **2(2)**, 31-37 **(2009)**.
[37] Ch.Serdevi, K.Balaj, A.Naidu, R.Sudhakaran; J.of Pharmacy Research, **2(9)**, 1500-1503 **(2009)**.
[38] P.A.Insel, in G.Goodman, A.G.Gilman, (Eds); 'The Pharmaceutical Basis of Therapeutics, Ninth Edition', Pergamon Press, Oxford, 638-681 **(1991)**.