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SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF NEW 2-ARYLIDENEHYDRAZINYL-QUINAZOLINONE AND 3-AMINO-TRIAZOLO-QUINAZOLINONE DERIVATIVES

Samy M. Ibrahim[*1], M. Abo-Kul[1], Moustafa K. Soltan[1], Ahmed S. Helal[1]

*[1]Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

ABSTRACT

A new series of 2-hydrazino-quinazolinone (III) and 3-amino-triazolo-quinazolinones (VII_{a-g}) were prepared upon hydrazinolysis of 2-mercapto-quinazolinone intermediates (II,VI_{a-g}) respectively. These compounds were condensed with aromatic aldehydes, for its confirmation; where compound (III) gives rise 2-(2-substitutedarylidenehydrazinyl)-3-(phenylamino) quinazolin-4(3H)-one (IV_{a-f}) and compound (VII_a) gives rise 3-(4-chloro benzylideneamino)-2-phenyl-[1,2,4] triazolo[5,1-b]quinazolin-9(3H)-one (X). The biological testing of the target compounds (IV_e, IV_f, VII_a, VII_f) showed moderate anti-inflammatory activity with low ulcerogenic index compared with diclofenac sodium and celecoxib.

KEY WORDS

2-hydrazino-quinazolinone, 3-amino-triazolo-quinazolinones and Anti-inflammatory.

Author of correspondence:
Samy M. Ibrahim,
Department of Medicinal Chemistry,
Faculty of Pharmacy,
Zagazig University, Zagazig, Egypt.

Email: dr-samy2010@hotmail.com.

INTRODUCTION

Hydrazinolysis is an important approach to the production of many heterocyclic compounds among of which hydrazinolysis of esters[1], amides[2], chloro- and thiol-derivatives[3, 4]. The idea of hydrazinolysis of two classes of 2-mercapto-quinazolinones; 2-Mercapto-3-(phenyl amino) quinazolin-4(3H)-one (II) and N-(2-Mercapto-4-oxoquinazolin-3(4H)-yl) substituted benzamide (VI_{a-h}), with different substituent in 3-position was arrived to determine whatever this substituent will affect the final product or not.

Many quinazolinone derivatives posses anti-inflammatory activity, among of which, Diproqualone (1), is a well known drug which used for the treatment of inflammatory pain associated with osteoarthritis[5]. The arylidene-amino- 4(3H) quinazolinone derivative (2) were synthesized and found to be exhibit anti-inflammatory activity[6].

From the previous information we prepare new series of 2-arylidenehydrazinyl-quinazolinone (IV_{a-f}) and 3-amino-triazolo-quinazolinone derivatives (VII_{a-g}) with the hope that these compounds may possess anti-inflammatory activity (Figure No.1).

EXPERIMENTAL PROTOCOLS

Chemistry

Melting points were determined with a Gallenkamp melting point apparatus (London, UK), and are uncorrected. IR spectra (KBr, cm-1) were recorded on a Bruker Vector, 22FT-IR Spectrometer (Bavaria, Germany). 1HNMR spectra were recorded on Varian Gemini-200 (200 MHz) Spectrometer (CA, USA) using DMSO-d6 as a solvent and tetramethylsilane (*TMS*) as an internal standard (Chemical shift in d, ppm). Electron impact mass spectra were determined at 70 eV using a GC/MS Shimadzu QP1000EXSpectrometer (Tokyo, Japan). Elemental analyses were determined using Heraeus or Vario EL-III (Elemntar) (Hanau, Germany) or Perkin Elmer Model 2400 (USA) CHN analyzers at the National Research Center and Micro analytical Center, Faculty of Science, Cairo University, Egypt. All the results of the elemental analyses were in an acceptable error range. Thin layer chromatography (TLC) was performed on silica gel G for TLC (Merck), and spots were visualized by irradiation with ultraviolet light (UV; 254 nm). All chemicals were purchased from Acros Chemicals (Belgium).

Preparation of 2-Amino- N'-phenyl benzohydrazide (I)

To a mixture of isatoic anhydride (8.5gm, 0.052mol) and phenyl hydrazine (5.6gm, 0.06mol) in ethanol (50ml), 10 drops of glacial acetic acid was added. The mixture was heated under reflux for 2 hours, concentrated to its half volume, and then cooled. The separated solid was filtered and crystallized from aqueous ethanol to give the titled compound (I) in 85% yield, m.p=175°C (as reported)[7,8].

Preparation of 2-Mercapto-3-(phenyl amino) quinazolin-4(3H)-one (II)

To a mixture of 2-amino-N'-phenylbenzohydrazide I (2.5gm, 0.011mol) and KOH (0.616, 0.011mol) in ethanol 95% (50 ml), carbon disulphide (0.837, 0.011 mol) was added. The reaction mixture was refluxed for 18hrs, then concentrated to half volume and poured into cold water (100ml). The separated solid was filtered and crystallized from ethanol to give compound (II) in 80% yield, m.p=258 - 261 °C (as reported)[9].

Preparation of 2-Hydrazinyl-3-(phenyl amino) quinazolin-4(3H)-one (III)

A mixture of 2-mercapto-3-(phenyl amino) quinazolin-4(3H)-one II (1 g, 0.0037mol) and hydrazine hydrate (0.187g, 0.011mol) in ethanol (50ml) was refluxed for 24 hour. The solvent was evaporated, the resultant residue was washed with water (50ml), dried and crystallized from ethanol / dioxane (1:1) to give white crystals Yield = 72%, m.p = 197-200°C the reported m.p.=175°C [10].

General procedure for preparation of 3-(Phenylamino)-2-(2-substitutedarylidene hydrazinyl) quinazolin-4 (3H)-ones (IV_{a-f})

To a mixture of 2-hydrazinyl-3-(phenyl amino) quinazolin-4(3H)-one (III) (1 gm, 0.0037mol) and the appropriate aldehyde (0.0037 mol) in ethanol (50 ml), few drops of acetic acid was added. The reaction mixture was refluxed for 2-4 hours, concentrated to its half volume, then cooled. The separated solid was filtered and crystallized from the appropriate solvent to give the titled compounds (IV_{a-f}).

2-(2-(4-(benzyloxy)benzylidene)hydrazinyl)-3-(phenylamino)quinazolin-4(3H)-one (*IV_a*)

Yield: 77 %; m.p. = 233-235 °C crystallized from ethanol/DMF,^{1}HNMR(200MHz):δ= 5.16 (s, 2H, Ar-CH_2O), 6.70-8.46 (m, 19H, Ar-H and -N=CH), 8.99(s, 1H, Ar-NH, exchangeable), 10.49(s, 1H, -C=N-NH, exchangeable), MS analysis:m/z (rel.intensity) = 462(M+1, 1.6),461(M^+, 4.6), 370(4.7), 236(5.4), 91(100), 77(16.8). Analysis for

$C_{28}H_{23}N_5O_2$ (461.51): Calcd: C, 72.87; H, 5.02; N, 15.17.Found: C, 72.71; H, 4.72; N, 15.17%.

2-(2-(4-chlorobenzylidene)hydrazinyl)-3-(phenylamino)quinazolin-4(3H)-one *(IV_b)*

Yield: 56.5 %; m.p. = 227-228 °C crystallized from ethanol. ^{1}HNMR(200MHz):δ= 6.71- 8.46 (m, 14H, Ar-H and -N=CH), 8.99 (s, 1H, Ar-NH, exchangeable), 10.67(s, 1H, -C=N-NH, exchangeable), MS analysis:m/z(rel.intensity)=390 (M+1, 7.5), 389(M^+, 19.2), 296((14.4), 236(14), 93(100), 77(56.2). Analysis for $C_{21}H_{16}ClN_5O$ (389.8): Calcd: C, 64.70;H, 4.14;N, 17.96 .Found: C, 64.62; H, 4.39; N, 17.90%.

2-(2-(3-chlorobenzylidene)hydrazinyl)-3-(phenylamino)quinazolin-4(3H)-one (IV_c)

Yield: 50 %; m.p. = 235-236°C crystallized from ethanol. MS analysis:m/z(rel.intensity)= 390 (M+1, 7.9), 389(M^+, 26.7), 297(15.5), 236(21.7), 93(100), 77(75.5). Analysis for $C_{21}H_{16}ClN_5O$(389.8): Calcd: C, 64.70;H, 4.14;N, 17.96.Found: C, 65.01; H, 4.28; N, 17.68%.

2-(2-(furan-2-ylmethylene)hydrazinyl)-3-(phenylamino)quinazolin-4(3H)-one (IV_d)

Yield: 37%; m.p. = 217 °C crystallized from dichloromethane/ether, ^{1}HNMR(200MHz):δ=6.45-8.30 (m, 13H, Ar-H and -N=CH), 8.92(s, 1H, Ar-NH, exchangeable), 9.66 (s, 1H, -C=N-NH, exchangeable), MS analysis:m/z (rel.intensity) =346(M+1, 13.9), 345(M^+, 60.8), 252(20.7), 236(31), 93(100), 77(76.2). Analysis for $C_{19}H_{15}N_5O_2$ (345.35): Calcd: C, 66.08;H, 4.38;N, 20.28.Found: C, 66.21; H, 4.51; N, 19.71%.

2-(2-(4-nitrobenzylidene)hydrazinyl)-3-(phenylamino)quinazolin-4(3H)-one (IV_e)

Yield: 68%; m.p. = 226-228 °C crystallized from ethanol, MS analysis:m/z(rel.intensity)= 401(M+1,7.5), 400(M+,17.8), 307(6.8), 236(12), 93(100), 77(41.1). Analysis for $C_{21}H_{16}N_6O_3$ (400.39): Calcd: C, 62.99;H, 4.03;N, 20.99.Found: C, 62.77; H, 4.10; N, 20.73%.

3-(phenylamino)-2-(2-(thiophen-2-ylmethylene)hydrazinyl)quinazolin-4(3H)-one (IV_f)

Yield: 40%;m.p.=204-207°C crystallized from dichloromethane/ether, MS analysis: m/z (rel.intensity) = 362(M+1,12.1), 361(M^+, 55), 269(12.8), 236(32.7), 93(100), 77(78.9). Analysis for $C_{19}H_{15}N_5OS$ (361.42): Calcd: C, 63.14;H, 4.15;N, 19.38.Found: C, 63.45; H, 4.30; N, 19.65%.

General procedure for preparation of 2-Amino-N'-substituted benzoyl benzo- hydrazides (V_{a-h})

A mixture of isatoic anhydride (8g, 0.049 mol) and the appropriate substituted benzoic acid hydrazide (0.049 mol) in ethanol (50 ml) containing10 drops glacial acetic acid was heated under reflux for 10-24 hours. The reaction mixture was cooled and the separated solid was filtered and crystallized from ethanol to give the titled compounds (as reported)[11].

General procedure for preparation of N-(2-Mercapto-4-oxoquinazolin-3(4H)-yl) substituted benzamides (VI_{a-h})

To a solution of 2-amino-N'-substituted benzoyl benzohydrazides V_{a-h} (0.0078mol) and potassium hydroxide (0.28 g, 0.0078mol) in ethanol 95% (50ml), carbon disulphide(0.6 g, 0.0078 mol) was added. The reaction mixture was heated under reflux for 18 hours, then concentrated to its half volume and then poured into cold water (100ml). The separated solid was filtered, dried and crystallized from ethanol to give the titled compounds (VI_{a-h}) (as reported)[12].

General procedure for preparation of 3-Amino-2-substituted phenyl-[1,2,4] triazolo [5,1-b] quinazolin-9(3H)-ones (VII_{a-g}) and N-(2-hydrazinyl-4-oxoquinazolin-3(4H)-yl)-4-nitro benzamide (VIII).

A solution of N-(2-mercapto-4-oxoquinazolin-3(4H)-yl) substituted benzamide (VI_{a-h}) (0.0067mol) and hydrazine hydrate (1g, 0.02mol) in ethanol (50ml) was heated under reflux for 24 hour. The solvent was evaporated; the obtained residue was washed with water (50 ml), dried and crystallized from ethanol/DMF(3:1) to give white or yellow crystals.

3-amino-2-phenyl-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (VII_a)

Yield: 82.2 %; m.p. = 282-286 °C crystallized from ethanol/DMF .^{1}HNMR(200MHz): δ=6.14 (s,2H,NH_2, exchangeable), 7.37-8.28(m,9H,Ar-H) ppm.MS analysis:m/z(rel.intensity)= 277.1 (M^+, 45.2),

262(77.4), 178(35.5), 145(51.6), 104(100), 90(58.1), 76(100). Analysis for $C_{15}H_{11}N_5O$ (277.28): Calcd: C, 64.97;H, 4.00;N, 25.26 Found: C, 64.81; H, 3.80; N, 25.46%.

3-amino-2-(4-chlorophenyl)-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (VII_b)

Yield: 68 %; m.p. = > 300 °C crystallized from ethanol/DMF**.** ^{1}HNMR(200MHz):δ=6.14 (s,2H,NH_2, exchangeable),7.37-8.24 (m,8H,Ar-H) ppm. MS analysis:m/z(rel.intensity) = 311($M^{+,}$ 52.3), 145(100), 90(34.4). Analysis for $C_{15}H_{10}ClN_5O$ (311.7): Calcd: C, 57.79;H, 3.23;N, 22.47.Found: C, 57.29; H, 3.18; N, 22.79%.

3-amino-2-(3-chlorophenyl)-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (VII_c)

Yield: 72 %; m.p. = 237-240 °C crystallized from ethanol/DMF**.** ^{1}HNMR(200MHz):δ=6.16 (s,2H,NH_2, exchangeable), 7.38-8.34 (m,8H,Ar-H) ppm. MS analysis:m/z(rel.intensity)= 312(M+1, 22.2), 295(100), 263(40.7), 145 (37.0), 90(48.1), 76(59.3). Analysis for $C_{15}H_{10}ClN_5O$ (311.7): Calcd: C, 57.79;H, 3.23;N, 22.47, Found: C, 57.50; H, 3.00; N, 22.59%.

3-amino-2-(3-nitrophenyl)-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (VII_d)

Yield: 63.5%; m.p. = > 300 °C crystallized from ethanol/DMF **.**^{1}HNMR(200MHz):δ=6.24 (s,2H,NH_2 ,exchangeable),7.38-9.15 (m,8H,Ar-H)ppm.MS analysis:m/z(rel.intensity) = 322(M^+, 4.0), 292(100), 145(43.2), 90 (34.4), 77(5.8). Analysis for $C_{15}H_{10}N_6O_3$ (322.28): Calcd: C, 55.90;H, 3.13;N, 26.08.Found: C, 55.71; H, 3.02; N, 26.44%.

3-amino-2-(4-methoxyphenyl)-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (VII_e)

Yield: 75.3 %; m.p. = 290-293 °C crystallized from ethanol/DMF **.**^{1}HNMR (200MHz):δ=3.87 (s,3H, OCH_3), 6.13 (s,2H, NH_2, exchangeable),7.15-8.26 (m,8H,Ar-H)ppm. MS analysis: m/z(rel.intensity) = 307(M^+, 69.6), 145(100), 90(55.8), 77 (28.1). Analysis for $C_{16}H_{13}N_5O_2$ (307.31): Calcd: C, 62.53;H, 4.26;N, 22.74.Found: C, 62.27; H, 4.10; N, 22.64%.

3-amino-2-(pyridin-4-yl)-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (VII_f)

Yield: 81.2 %; m.p. = > 300 °C crystallized from ethanol/DMF ^{1}HNMR(200MHz):δ=6.20 (s,2H,NH_2, exchangeable), 7.41-8.88 (m,8H,Ar-H) ppm. MS analysis:m/z(rel.intensity) = 278(M^+, 41.2), 263(72.1), 145(39.7), 120(41.2), 104(85.3), 90(36.8), 76.1(100). Analysis for $C_{14}H_{10}N_6O$ (278.27): Calcd: C, 60.43;H, 3.59;N, 30.20.Found: C, 60.65; H, 3.33; N, 29.99%.

3-amino-2-(pyridin-3-yl)-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (VII_g)

Yield: 71 %; m.p. = > 300 °C crystallized from ethanol/DMF ^{1}HNMR(200MHz):δ=6.17 (s,2H,NH_2, exchangeable),7.36-9.29 (m,8H,Ar-H) ppm. MSanalysis:m/z(rel.intensity) = 278(M^+, 30), 145(50.2), 102(45.9), 90(37.7),77(49). Analysis for $C_{14}H_{10}N_6O$ (278.27): Calcd: C, 60.43;H, 3.59;N, 30.20.Found: C, 60.02; H, 3.53; N, 30.29%.

N-(2-hydrazinyl-4-oxoquinazolin-3(4H)-yl)-4-nitrobenzamide (VIII)

Yield: 57.4 %; m.p. = > 300 °C crystallized from ethanol/DMF ^{1}HNMR**(**200MHz):δ=6.20 (s,2H,NH_2), 7.38-8.40 (m,8H,Ar-H), 11.89 (s, 1H, amidino-NH), 13.25 (s,1H, CONH-) ppm. MS analysis: m/z(rel.intensity) = 342(M+2, 28.3), 341(M+1, 12.7), 340(M^+,5.5), 309(16.4), 150(100), 104(56.7), 76(52.0). Analysis for $C_{15}H_{10}N_6O_3$ (340.29): Calcd: C, 52.94;H, 3.50;N, 24.70.Found: C, 52.81; H, 3.00; N, 24.51%.

3-(4-Nitrobenzylideneamino)-2-(4-nitrophenyl)-[1,2,4]triazolo[5,1-b] quinazolin-9(3H)-one (IX)

To a solution of N-(2-hydrazinyl-4-oxoquinazolin-3(4H)-yl)-4-nitrobenzamide (VIII), (1gm, 0.003 mol) in ethanol 20ml acidified with 10 drops acetic acid, p-nitrobenzaldehyde (0.45 gm, 0.003 mol) was added and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled and the separated solid was filtered and crystallized from DMF / ethanol.

Yield: 74.6 %; m.p. = > 300 °C crystallized from ethanol/DMF. ^{1}H NMR (200MHz) δ= 6.24-8.54 (m,12H,Ar-H), 8.55 (s,1H,N=CH) ppm. MSanalysis:m/ z(rel.intensity) = 455(M^+, 13.4), 307(56.6) , 148(30.9), 102(100), 90(30.3), 76(57.7). Analysis for $C_{22}H_{13}N_7O_5$ (455.38): Calcd: C,

58.02;H, 2.88;N, 21.53.Found: C, 58.40; H, 2.53; N, 22.03%.

3-(4-Chlorobenzylideneamino)-2-phenyl-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (X)

To a solution of 3-amino phenyl-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one VII_a (0.831gm, 0.003 mol) in ethanol 20ml acidified with acetic acid, p-chlorobenzaldehyde (0.42 gm, 0.003 mol) was added and the reaction mixture was refluxed for 2 hours. The reaction mixture was cooled and the separated solid was filtered and crystallized from DMF / ethanol.

Yield: 63.5 %; m.p.= 267-270°C crystallized from ethanol/DMF**.** MS analysis:m/z (rel.intensity) = 399(M^+, 68.0), 275(41.0), 259(75.33), 145(45.33), 139(44.0), 122(42), 7(38.67).

Analysis for $C_{22}H_{14}ClN_5O$ (399.83): Calcd: C, 66.09;H, 3.53;N, 17.52.Found: C, 65.99; H, 3.41; N, 17.80%.

Pharmacology

Male albino rats and male mice, weighing 150-200 gm and 20-25 gm each, respectively were obtained from the Laboratory Animal Services Center, Faculty of veterinary Medicine, Zagazig University, Zagazig, Egypt. The animals were maintained on a 12-h light/dark cycle under regulated temperature (25 ± 2 °C) and humidity (50 ± 10 %) as well as fed with standard diet and water ad libitum. They were allowed to acclimate 7 days before use. This protocol was approved by the Animal Care and Use Committee of the Pharmacology department, Faculty of veterinary Medicine, Zagazig University.

Acetic-acid-induced writhing

The test was carried out using the previously described technique Elisabetsky[13]. Mice were divided into 6 groups each of 5 mice and were injected intraperitoneally (i.p) with 0.1 ml/10 g body weight of 0.6% acetic acid solution in normal saline 1 h after the oral administration of tested compounds (IV_e, IV_f, VII_a, VII_f) at a dose of 50 mg/kg. The frequency of writhing was recorded within 25 min from the injection of acetic acid. Celecoxib was administrated to one group of mice at a dose level of 50 mg/kg as a positive control. One group of mice was lefted as a control.

Carrageenan-induced paw edema

The effects of test compounds (IV_e, IV_f, VII_a, VII_f) on rat paw edema induced by carrageenan **were** studied as described by Winter[14]. The substances were tested at 50 mg/kg. Test substances were suspended in gum acacia (7% suspention) while diclofenac sodium was dissolved in hot distilled water. Diclofenac sodium was tested at 5 mg/kg. The diameter of the right paw of each animal was determined using a micrometer. The test substances were administered by means of oral administration. The control group received only the corresponding vehicle. Thirty minutes later, paw edema was induced by subcutaneous injection of 0.1 ml carrageenan (0.1%) into the subplantar surface of the right hind paw of all animals. The paw diameter was measured 1, 2, 3, 4, 5 and 24 h after the injection. The AUC relating variation of edema to time was obtained using the trapezoidal rule[15]. Total inhibition (TI, %) was obtained for each group and at each record, using the following ratio: TI (%)=[AUC control −AUC treat]×100/AUC control. Data were expressed as mean ± standard error of mean (SEM) of 5 animals.

Ulcerogenic activity

Male albino rats weighing 150-200 gm were fasted for 12 hours prior to drug administration. Water was supplied ad-libitum. The animals were divided into 7 equal groups (each of 5). The first group received 7% gum acacia (suspending vehicle) orally once a day and left as a control, whereas, the second and third groups received indomethacin and celecoxib (as refrence drugs) at a dose of 18 and 50 mg/ kg/ day orally, respectively. Groups from 4^{th} to 7^{th} received the tested compounds (IV_e, IV_f, VII_a, VII_f) at 50 mg/ kg/ day. The tested compounds were administered once a day for 3 successive days. The animals were killed by overdose of ether 6 hours after the last dose. The stomachs were removed, opened along the greater curvature and examined for ulceration. The number and diameter of discrete areas of damage in the glandular mucosa were scored. The ulcer score was calculated according to the 1 to 10 scoring system of Valcavi[16].

RESULTS AND DISCUSSION

Chemistry

In this study, two starting compounds (II, VI_{a-h}) were synthesized to be conducted into hydrazinolysis procedures.

The first thiol-intermediate (II); 2-mercapto-quinazolinone which carry aniline-moiety in 3-position, upon hydrazinolysis, gives the expected 2-hydrazinyl-3-phenylamino-quinazolin-4(3H)-one (III) which was confirmed by condensation with different aromatic aldehydes to give the final compounds (IV_{a-f}). The thiol-intermediate (II) was firstly prepared through condensation of isatoic anhydride with phenyl hydrazine followed by cyclization with carbon disulfide/potassium hydroxide. This is illustrated in Figure No.2.

Because the founded melting point of 2-hydrazinyl-quinazolinone (III)(197-200℃) differs from the reported one (175°*C*)[10], IR, ^{1}HNMR and mass spetra were done to confirm the chemical structure of this intermediate (III). Where IR spectrum showed the appearance of the absorption bands at ν = 3562, 3425 and 3333 $cm^{-1}$for amidino-NH, Ar-NH and NH_2 respectively. In addition to a strong absorption bands at ν = 1670 cm^{-1} for the amidic carbonyl. ^{1}HNMR data revealed the appearance of three singlets at = 8.81, 8.59 and 4.37 ppm for amidino-NH, Ar-NH and NH_2 protons respectively. While the mass spectrum showed the fragment of aniline (93,100%) in addition to the molecular ion peak (267,7.3%), the molecular weight of the intermediate ; 2-hydrazinyl-3-(phenyl amino) quinazolin-4(3H)-one (III).

The structures of the final compounds (IV_{a-f}) were confirmed by the IR, ^{1}HNMR and mass spectra. ^{1}HNMR spectrum of p-chlorobenzylidene-hydrazino-quinazolin-4(3H)-one (IV_b) for example, showed the disappearance of the singlet peak at δ = 4.37ppm associated with NH_2-protons in the key intermediate (III), and the presence of two singlets at δ =11.240 ppm and δ = 10.67 ppm due to amidino-NH and Ar-NH protons respectively. The mass spectra are good tool to determine the molecular weights of the final compounds; arylidenhyrazino-quinazolin-4(3H)-ones (IV_{a-f}), in addition to the characteristic neutral aniline fragment of m/z = 93 with 100% intensity, as well as the fragment of m/z = 236 (with 21.7% intensity in IV_c, 31% intensity in IV_d, 32.7% intensity in IV_f) which formed upon removal of arylideno-hydrazine fragment from the target compounds(IV_c, IV_d, IV_f).

The second thiol-intermediates carrying benzamide moiety in the 3-position; N-(2-mercapto-4-oxoquinazolin-3(4H)-yl) substituted benzamide (VI_{a-h}), upon hydrazinolysis gives rise fused heterocyclic compounds; 3-amino-2-substituted phenyl-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (VII_{a-g}). The thiol-intermediates were prepared firstly by condensation of isatoic anhydride with the appropriate benzoic acid hydrazide followed by cyclization of the produced benzamides (V_{a-h}) with carbon disulfide/potassium hydroxide. This is illustrated in Figure No.3.

The expected result of hyrazinolysis of N-(2-mercapto-4-oxoquinazolin-3(4H)-yl) substituted benzamide (VI_{a-h}) is the replacement of the thiol moiety with hydrazine one, but the presence of the terminal benzamide carbonyl shift the reaction to the formation of 3-amino-triazolo [5,1-b]quinazolin-9(3H)-ones (VII_{a-g}). This reaction is postulated as in Figure No.4.

The 2-hydrazino-quinazolinone derivative (VIII) is separated only in the case of 4-nitrobenzamide-quinazolinone intermediate (VI_h) upon reaction with hydrazine hydrate. This compound was confirmed by condensation with p-nitrobenzaldehyde in acidic medium, and the amazing result is the formation of 3-(4-nitrobenzylideneamino)-2-(4-nitrophenyl)-[1,2,4] triazolo [5,1-b]quinazolin-9(3H)-one (IX) rather than 2-arylidinhydrazino-quinazolinone derivative. This reaction is illustrated in Figure No.5.

The chemical structures of the final compounds; 3-amino-2-substituted phenyl-[1,2,4] triazolo[5,1-b]quinazolin-9(3H)-ones (VII_{a-g}) were confirmed by the elemental analysis, in addition to IR, ^{1}HNMR and mass spectra. Where IR spectrum of (VII_a) as example predicts one carbonyl at 1690 cm^{-1}indicating ring closure and formation of the fused triazolo-quinazolinone derivatives (VII_a). The IR of compound (VIII) contains two carbonyls at

1689 & 1643 cm^{-1} indicating the 2-hydrazino-quinazolinone derivative (VIII). ^{1}HNMR spetra of compounds (VII_{a-g}) showing singlet peaks at δ = 6.14, 6.14, 6.16, 6.24, 6.12, 6.20 and 6.17 ppm for NH_2 protons in the compounds VII_a, VII_b, VII_c, VII_d, VII_e, VII_f and VII_g respectively. On the other hand, the ^{1}HNMR spectrum of compound VIII shows three singlets at δ = 13.25, 11.89 and 6.20 ppm for amidic-NH, amidine-NH and NH_2 protons respectively.

Mass spectra are still a convenient tool to confirm the chemical structure of the final compounds; 3-amino-2-substituted phenyl-[1,2,4] triazolo[5,1-b]quinazolin-9(3H)-ones **(**VII_{a-g}**)**, where the molecular ion peaks indicating the molecular weights of the cyclized triazolo-quinazolinones rather than the non-separated hydrazine-quinazolinones. Splitting the amino group from the compounds; VII_c, VII_f and VII_a left fragments of m/z = 295(100), 263(72.1) and 262(77.4) respectively, which confirm the fused triazolo-quinazolinone structure of these compounds. Also removal of (N-NH_2) biradical from compounds; VII_d and VII_e left fragments of m/z = 292(100) and 277(52.7) of 3-(3-nitrobenzylidene-amino)quinazolin-4(3H)-one and 3-(4-methoxybenzylidene amino) quinazolin-4(3H)-one respectively.

On the other hand mass spectrum of compound VIII shows the molecular ion peak of (m/z = 340) which exceeds by water molecule over that of the m-nitro-isomer VII_d (m/z = 322) produced by the same procedure. Removal of ($NHNH_2$) radical left a frgment of (m/z = 309) for 4-nitro-N-(4-oxoquinazolin-3(4H)-yl)benzamide radical, this an indication that compound VIII is a hydrazine-quinazolinone derivative.

Also, the condensation product; 3-(4-Nitrobenzylideneamino)-2-(4-nitrophenyl)-[1,2,4] triazolo[5,1-b] quinazolin-9(3H)-one (IX), was confirmed by elemental analysis, ^{1}HNMR and mass spectra.

The synthesis of the final compound; 3-(4-Chlorobenzylideneamino)-2-phenyl-[1,2,4] triazolo[5,1-b]quinazolin-9(3H)-one (X) was done as an extra confirmatory tool of the separated 3-amino-triazoloquinazolinones (VII_{a-g}). Condensation of 3-Amino-2-phenyl-[1,2,4]triazolo[5,1-b]quinazolin-9(3H)-one (VII_a) with p-chloro-benzaldehyde in ethanol acidified with acetic acid, was done to achieve the final target(X). The chemical structure of the final compound (X) was confirmed by its elemental analysis as well as mass spectrum. The molecular ion peak in the mass spectrum [399(M^+, 68.0)] revealed the molecular weight of this compound.

Pharmacology

The newly synthesized 2-arylidenehydrazinyl-quinazolinones (IV_e, IV_f)and 3-amino-triazolo-quinazolinone derivatives (VII_a, VII_f) were tested for their analgesic activity using acetic-acid-induced writhing method in mice[13] using celecoxib as a reference , anti-inflammatory using Carrageenan-induced paw edema[14] using celecoxib and diclofenac sodium as reference drugs. Also these compounds tested for their ulcerogenic effect[16].

Analgesic activity

In this work we explored the moderately active compounds with lesser side effects in comparison with reference drugs. The results present in (Table No.1) revealed that compound IV_e showed analgesic activity approximately equal to that of celecoxib by the reduction in the total number of writhing induced in mice by IP injection of acetic acid. Other tested compounds showed mild
to moderate analgesic activity.

Anti-inflammatory activity

It was observed from (Table No.2) that compounds (IV_e, IV_f ,VII_a, VII_f) were taken as oral pretreatment at a dose of 50 mg/kg ; and these compounds significantly decreased rats hind paw edema thickness. Results obtained from AUC The calculation show that: The rank order of potency was: diclofenac sodium > celecoxib > IV_e > VII_f > VII_a> IV_f. It is noticed that exchange of thiophene ring in compound IV_f with furan ring in compound IV_e results in increase of activity. Also replacement of phenyl ring in compound VII_a by 4-pyridyl ring in compound VII_g increase activity.

Ulcerogenic activity

The novel compounds (IV_e, IV_f, VII_a, VII_f) were tested for their analgesic, anti-inflammatory and

ulcerogenic activity using celecoxib and diclofenac sodium for comparison and as a reference for the ulcerogenic activity (Table No.3).

The ulcerogenic activity study revealed that all the tested compounds showed non-significant ulcerogenic activity in comparison to the celecoxib. Administration of diclofenac sodium resulted in obvious ulcers in all tested animals while celecoxib showed less ulcerogenic activity. Furthermore, compound VII_f didn't show any ulcergenic activity.

Table No.1: Analgesic activity of compounds IV_e, IV_f, VII_a, VII_f administered in a dose of 50 mg/kg against acetic acid-induced writhing

S.No	Treatment	Writhing					Total	% reduction
		Time interval (minutes)						
		0-5	5-10	10-15	15-20	20-25		
1	Control	28.5 ± 1.9	21 ± 1.8#	18 ± 0.8	22.2 ± 0.8#	20.75± 0.8#	110.5	0
2	Celecoxib	32 ± 1	11.5 ± 0.6*	14.7 ± 1.6	8.75 ± 0.8*	6 ± 0.9*	73	33.9
3	IV_e	13.6 ± 2.1*#	21 ± 0.5#	20 ± 4.3	15.3 ± 2.5*#	6.7 ± 8.1*#	69.6	30.4
4	IV_f	23 ± 2.9#	18 ± 1.5#	20 ± 3.2	14.9 ± 1.8*#	12.1 ± 1.1*	79.5	20.5
5	VII_a	19.2 ± 1.9*#	16.4 ± 1.5#	17.3 ± 3.2	16.3 ± 1.8*	11.5 ± 1.1*	73	27.1
6	VII_f	16.6 ± 2.9*#	22.4± 1.5#	25.7 ± 3.2	17.1 ± 1.8*#	8 ± 1.1*#	77	23.0

* Significantly different from control group at $p<0.05$.
# Significantly different from celecoxib group at $p<0.05$.

Table No.2: Anti-inflammatory activity of compounds IV_e, IV_f, VII_a, VII_f, at a dose level of 50 mg/kg against rat hind paw edema induced by carragennan

S.No	Time (hr)	Carrageenan	Celecoxib	Diclofenac sodium	IV_e	IV_f	VII_a	VII_f
1	0	100	100	100	100	100	100	100
2	1	155±2.6	141±6.3	130±4.6	127.26±1.6	126.88±2.9	126.25±1.5	126.25±1.5
3	2	164±6.6	122±0.9*	115.9±1.1*	124.22±2.5	130.4±2.4	122.67±1.6	122.31±1.5
4	3	160±6.7	112±1.8*	111.14±2*	114.07±2.5*	123.08±1.9*	112.86±1.6*	112.50±1.5*
5	4	151± 4.9	108.8±1.7*	108.6±3.6*	107.29±2.4*	115.25±1.2*	106.54±1.5*	106.32±1.4*

6	5	144±5	104.3±0.7*	103.49±1.5*	102.21±1.1	107.97±1.6	104.17±1.3	103.58±1
7	24	1036± 2.2	96.6±1.4	99.18±5	100.74±0.9	101.67±1.8	101.7±1.3	99.96±1.6
8	AUC	3099	2495	2493	2502	2591	2526	2503
9	% reduction	0	20.45	20.51	20.21	17.20	19.70	20.17

* Significantly different from 1 hr time point within the same group at $p<0.05$.
Data expressed as mean ± SEM from six different experiments; *: $p < 0.05$ compared with carrageenan.

Table No.3: Ulcerogenic activity of test compounds IV_e, IV_f, VII_a, VII_f, diclofenac sodium and celecoxib (n=5)

S.No	Treatment	Ulcer index
1	Indomethacin	28.6 ± 0.7
2	Diclofenac sodium	8.9 ± 0.7
3	Celecoxib	4.7 ± 0.3 *
4	IV_e	2.1 ± 0.1 * #
5	IV_f	1.0 ± 0.1 * #
6	VII_a	0.6 ± 0.1 * #
7	VII_f	0.02 ± 0.0 * #

* Significantly different from diclofenac sodium group at $p<0.05$.
# Significantly different from celecoxib group at $p<0.05$.

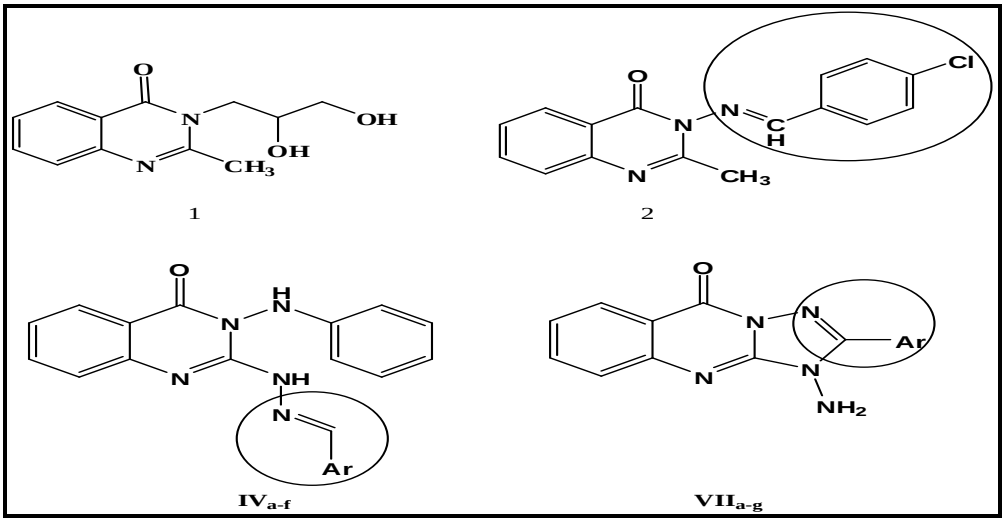

Figure No.1: Quinazolinones derivatives compounds

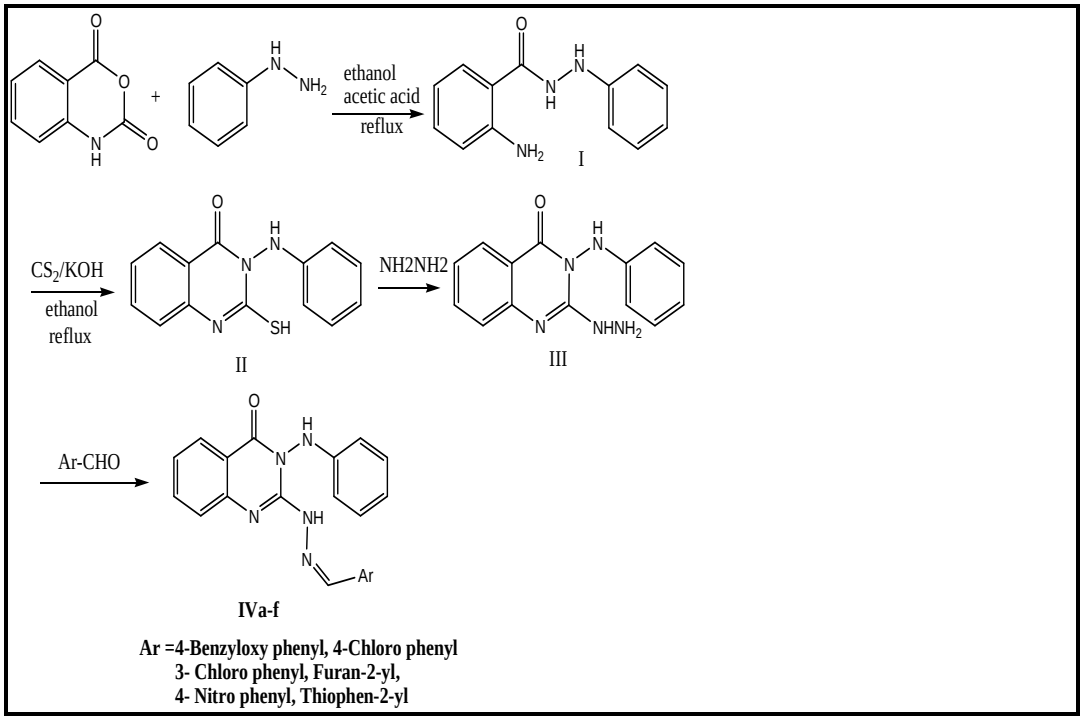

Figure No.2: Synthetic pathway for compounds IV_{a-f}

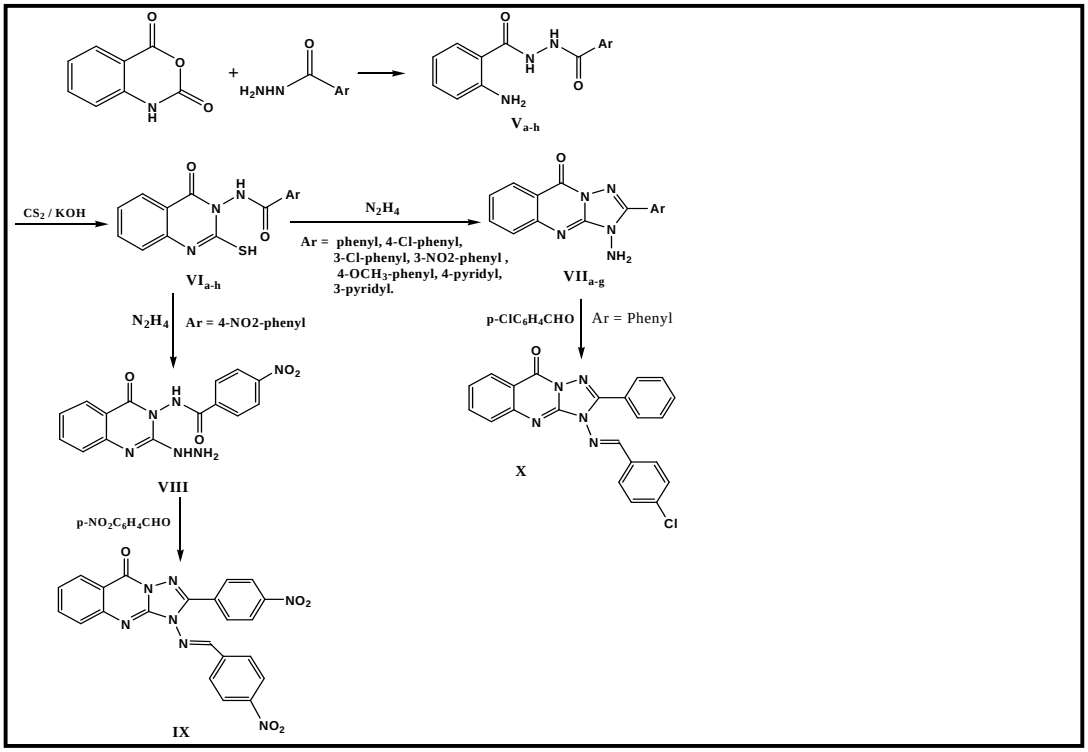

Figure No.3: Synthetic pathway for compounds (VII_{a-g}), (VIII), (IX), (X)

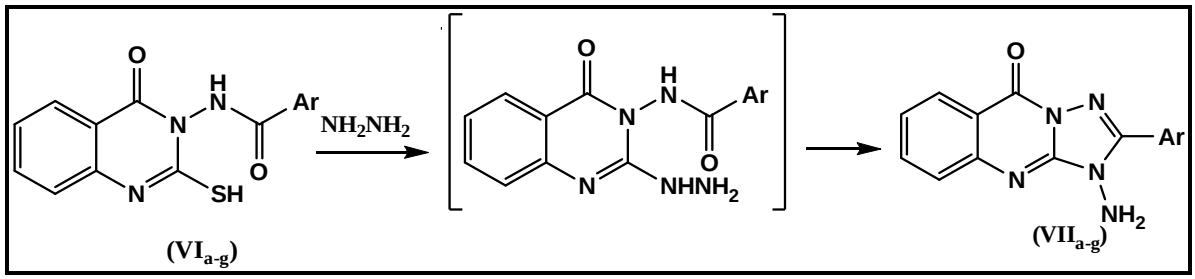

Figure No.4: The mechanistic pathway for hydrazinolysis of (VI_{a-g})

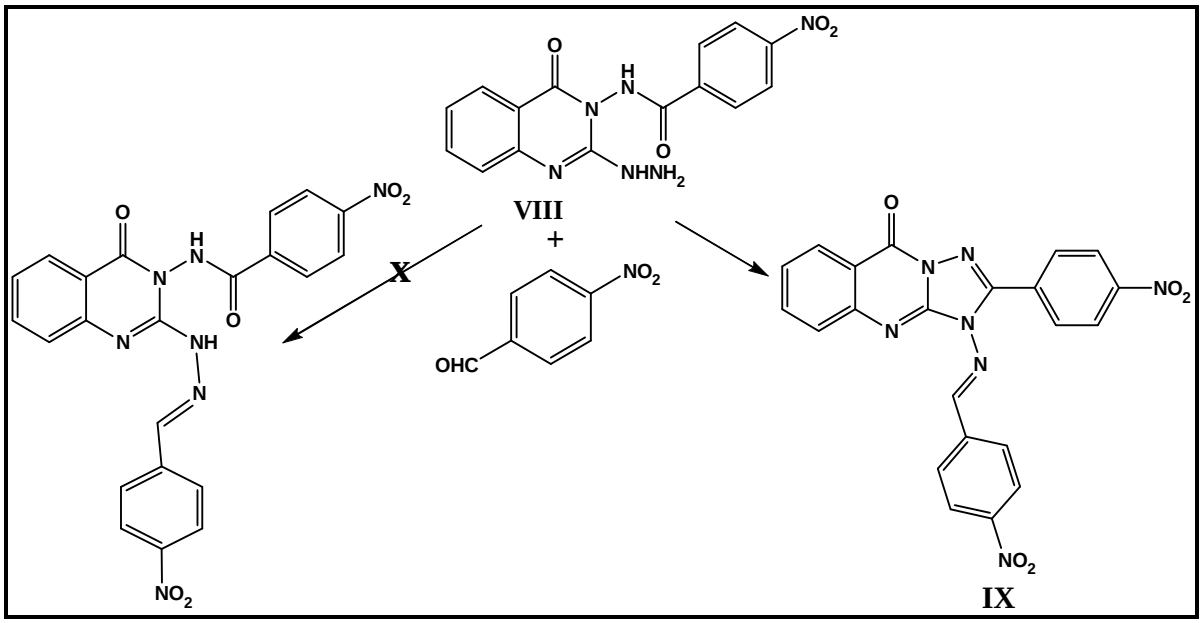

Figure No.5: Reaction of (VIII) with p-nitrobenzaldehyde in acidic medium

CONCLUSION

Our conclusion herein is the achievement of an excellent synthetic pathway of new series of 2-arylidenehydrazinyl-quinazolinone derivatives and 3-amino-triazolo-quinazolinones in an easy and concise way. The amazing unexpected condensation product (IX) from the intermediate (VIII) may open the approach to new series of 3-arylidene-amino-triazolo-quinazolinones and other derivatives. In the pharmacological screening, it was found that newly synthesized compounds (IV_e, IV_f, VII_a, VII_f) exhibited analgesic and anti-inflammatory activities with non significant ulcerogenic effect.

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