STRUCTURE ANTI-INFLAMMATORY ACTIVITY RELATIONSHIP AND BIOCHEMICAL EVALUATION OF SOME NOVEL TRIAZOLOQUINAZOLINE AND TRIAZINOQUINAZOLINE DERIVATIVES CONTAINING SULFACETAMIDE MOIETY

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ABSTRACT-: This study was carried out to evaluate the structure activity relationship and biochemical effects of some novel of anti-inflammatory triazoloquinazolines and triazinoquinazolines containing sulfacetamide moiety. Some of the newly synthesized compounds 4-17 showed good anti-inflammatory activities. Also, the median lethal doses (LD_{50s}) of compounds 8, 10 and 17 in mice were 370, 338 and 295 mg/100g b.w., respectively. Oral administration of compounds 8, 10 and 17 to the rats at dose of 300 mg/kg.b.w. for 10 days showed non-significant changes in liver enzymes SGOT, SGPT, ALP, LDH, γ –GT, SOD and GPx and blood GSH and serum TBARs as compared with the control group. But, administration of indomethacin orally to the rats at a concentration of 600 mg/kg b.w daily for 10 days to rats showed significant increase in serum SGOT, SGPT, ALP, LDH, γ –GT and TBARs and significant decrease in blood GSH, SOD and GPx. These findings suggest that compounds 8, 10 and 17 exhibited good anti-inflammatory activity and more safe on rat liver enzymes.

Keywords-: Anti-inflammatory, Quinazoline, sulfacetamide, LD_{50}, SGOT, SGPT, ALP, LDH, γ –GT, SOD, GPx , blood GSH and TBARs.

INTRODUCTION

Quinazolines and 1,2,4-triazoles have been reported to have several biological activities[1]-[6]. Combining these two structure features in one molecule might produce compounds with promising biological effects [7]. The potential activity of quinazolines as antitumor [8], [9], anticancer [10], [11] and antimicrobial activities [12], [13] is well known. These reports prompted me to investigate the other physiological and pharmacological functions of triazoloquinazoline, triazinoquinazoline. In this study, I sought to evaluate the structure activity relationship and biochemical effects of some novel of anti-inflammatory triazoloquinazolines and triazinoquinazolines containing sulfacetamide moiety. In addition, the acute toxicity and biochemical effects of anti-inflammatory compound **8**, **10** and **17**evaluated in order to assess its safety.

EXPERIMENTAL

Chemistry

Melting points were determined on Gallen-kamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on shimadzu MR 470 infrared spectrophotometer using the KBr pellets. ^{1}HNMR spectra were recorded on a Varian EM 360 (240 MHz) instrument using TMS as an internal standard (chemical shift in δ ppm(. Microanalytical data (C, H, N) were determined at the Microanalytical centre, Cairo University, Egypt. Mass spectra were run using HP Model MS-5988.

Methyl-2-(4-N-substituted-sulfamoyl) phenylthioureido benzoate (3)

Method (A):

To a solution of methyl-2-isothiocyanato benzoic acid **2** (0.01 mol) in dimethylformamide (20 ml), sulfacetamide (0.01 mol) were added. The reaction mixture was stirred under room temperature for 3-4h furnished the thioureido derivative **3**. The crude product was recrystallized from ethanol to give **3**.

Method (B):

Methyl -2-(4-N-subistuted sulfamoyl)phenyl thioureido benzoate **3** was prepared by adding carbondisulphide (0.01 mol) and sodium hydroxide solution (0.01 mol) simultaneously to a vigorously stirred of sulfacetamide (0.01 mol) in dimethylsulphoxide during 30 min, stirring was continued for 30 min more. Dimethyl sulphate (0.01 mol) was added to the reaction mixture with stirring at 5-10°, it was further stirred for 2 h and poured into ice water to get a semi solid methyl[4-(acetylsulfamyl)phenyl]dithiocarbamate **2'**. The compound **2'** (0.01mol) and methyl anthranilate (0.01mol) were stirred in dimethylformamide (20 ml) under room temperature for 3-4h furnished the thioureido derivative **3**. The crude product was recrystallized from ethanol to give**.** IR(KBr, cm^{-1}) **3**: 3471,3317(NH), 3100(CH-arom.), 2916, 2819(CH-aliph.), 1701,1678(2C=O), 1375, 1175 (SO_2), 1299(C=S).^{1}H-NMR (DMSO-d_6) **3**: 2.4 [s, 3H, $COCH_3$], 3.6 [s, 3H, OCH_3], 7.3 – 8.4 [m, 10 H, Ar-H + 2 NH], 13.06 [s, 1H, SO_2NH].

3-amino-2-(4-methylaminomethyl-phenylamino)-3H-quinazolin-4-one (4).

A mixture of **3** (0.01 mol) and hydrazine hydrate (0.02 mol) in n-butanol (30 ml) was refluxed for 5h. After cooling, the reaction mixture was poured onto ice water and the solid obtained was filtered off and recrystallized from dioxane to give **4**. Physicochemical and analytical data are listed in table 1. IR (KBr, cm^{-1}) **4**: 3460, 3348, 3251(NH, NH_2), 1687, 1650 (2C=O), 1610(C=N) 1315, 1188 (SO_2), 1257(C=S). MS (m/z)**4**: 377 (M^++ 4, 59.8%), 304(100%), 331(33.6%),76(36.1%), 52(22.1%).

Table 1: Physico-chemical properties and molecular formulae of the synthesized compounds

.Compd .No	[M.P. [°C	Yield (%)	Mol. Formula (.Mol. Wt)	Elemental analyses [%] Calcd./Found		
				C	H	N
3	>300	69	$C_{17}H_{17}N_3O_5S_2$ (407)	50.12 50.34	4.17 4.30	10.31 10.30
4	217-219	79	$C_{16}H_{15}N_5O_4S$ (373)	51.47 51.30	4.02 4.23	18.76 18.44
5	> 300	68	$C_{23}H_{17}N_5O_4S$ (459)	60.13 60.34	3.70 3.35	15.25 15.40
6	>300	92	$C_{23}H_{17}N_5O_5S$ (475)	58.10 58.27	3.57 3.44	14.73 14.61
7	>300	83	$C_{23}H_{16}N_5O_4SCl$ (493)	55.98 55.77	3.24 3.13	14.19 14.31
8	> 300	76	$C_{25}H_{22}N_6O_4S$ (502)	59.76 59.59	4.38 4.30	16.73 16.85
9	225-227	80	$C_{18}H_{15}N_5O_4S$ (397)	54.40 54.70	3.77 3.90	17.63 17.40
10	91 – 92	88	$C_{23}H_{20}N_6O_4S_2$ (508)	54.33 54.66	3.93 3.81	16.53 16.34
12	221 -223	72	$C_{18}H_{15}N_5O_5S$ (413)	51.00 51.17	3.63 3.40	16.94 16.60
17	240-242	65	$C_{21}H_{19}N_5O_6S$ (469)	53.73 53.31	4.05 3.24	14.92 14.11

N-Acetyl-4-(9-oxo-2-substituted-9H-[1,2,4]triazolo[5,1-b]quinazolin-3-yl)-benzenesulfonamide (5-8).

A mixture of **4** (0.01 mol) and the appropriate aromatic aldehydes (0.01 mol) in glacial acetic acid (50 ml) containing fused sodium acetate (0.5g) was heated under reflux for 4hr. The solvent was concentrated and the reaction mixture was poured onto ice water. The obtained solid was recrystallized from dioxane to give **(5-8)**, respectively. Physicochemical and analytical data are listed in table 1. IR (KBr, cm^{-1}) **5:** 3264(NH), 3058(CH-arom.), 2940, 2840(CH-aliph.), 1672, 1598(2C=O), 1598(C=N) 1308, 1156(SO_2). MS (m/z) **5**: 431(M^+-CO, 12.50%), 429(10.7%), 393(5%), 338(22%), 321(2.7%), 287(27.3%), 194(100%). IR (KBr, cm^{-1}) **6:** 3446 (OH), 3290(NH), 3100(CH-arom.), 2960, 2836(CH-aliph.), 1622, 1650(2C=O), 1622(C=N), 1316, 1156(SO_2). MS (m/z) **6**:458(M^+-OH,3.08%), 287(100%), 228(20.5%), 194(60.9%), 167(5.4%), 117(4.2%), 77(10%). IR (KBr, cm^{-1}) **7:** 3452(NH), 3061(CH-arom.), 2950, 2827(CH-aliph.), 1685(2C=O), 1593(C=N), 1315, 1141(SO_2). MS(m/z) **7**: 493 (M^+,1.67%), 236 (8.53%),207(7.15%),155 (44.17%),139 (100%),140(28%), 115(4.7%), 100(6.6%), 51(11%). IR (KBr, cm^{-1}) **8:**3436(NH), 3066(CH-arom.), 2940, 2836(CH-aliph.), 1735, 1642(2C=O), 1560(C=N), 1324, 1136(SO_2). ^{1}H-NMR (DMSO-d_6) **8**: 2.3(s, 6H, $2CH_3$), 2.9(s, 3H, $COCH_3$), 7.0-7.8(m, 13H, Ar-H+NH), 10.7[s, 1H, SO_2NH_2].

N-Acetyl-4-(2-methyl-9-oxo-9H-[1,2,4]triazolo[5,1-b]quinazolin-3-yl) benzenesulfonamide (9).

A solution of **4** (0.01 mol) in acetic anhydride (20 ml) was refluxed for 8 hr. The solvent was concentrated and the residue was recrystallized from dioxane to give **9**. Physicochemical and analytical data are listed in table 1. IR (KBr, cm^{-1}) **9**:3416 (NH), 1674(2C=O), 1596(C=N), 1314, 1158 (SO_2). MS(m/z)**9**: 382(M^+-CH_3,7.8%), 341(100%), 331(37.4%), 277(42.6%), 262(35%), 234(20%), 206(16%), 161(10%), 103(5%), 76(2.9%).

N-acetyl-4-[4-oxo-3-(3-phenylthioureido)-3,4-dihydro-quinazoline-2-yl-amino]benzenesulfonamide (10).

A mixture of **4** (0.01 mol) and phenyl isothiocyanate (0.01 mol) in ethanol (20 ml) was refluxed for 8 hr. The solvent was then concentrated and the residue was recrystallized from ethanol to give **10**. Physicochemical and analytical data are listed in table 1. IR (KBr, cm^{-1}) **10:** 3382, 3290(2NH), 3054 (CH-arom.), 1670, 1586(2C=O), 1542(C=N), 1316, 1130(SO_2). MS (m/z) **10**: 508 (M^+, 0.4%), 327 (10.5%), 288 (22.5%), 287 (100%), 194 (72.2%), 167(3.1%), 150 (2.7%), 93(2%), 77(4%), 65(1.7 %).

N-Acetyl-(4-(3,10-dioxo-1,2,3,10-tetrahydro-1H -[1,2,4]triazino[6,1-b]quinazolin-4 (10H)-yl) phenylsulfonyl)acetamids(12).

A mixture of **4** (0.01 mol) and ethyl chloroacetate (0.01 mol) in methanol containing sodium methoxide (0.23 g Na in 15 ml CH_3OH) was refluxed for 10 hr. After cooling, the reaction mixture was poured onto ice water and the solid obtained was filtered off and recrystallized from acetic acid to give **12**. Physicochemical and analytical data are listed in table 1. IR (KBr, cm^{-1}) **12**: 3390, 3340 (2NH), 3035(CH-arom.), 2950, 2865(CH-aliph.), 1690, 1662, 1624(3C=O), 1350, 1199(SO_2), MS (m/z) **12**: 413(M^+, 2.1%), 199(100%), 271.67(33.6%), 214.02 (21.4%), 155(43%), 120(5%), 92(6%), 65.13(2.2%),^{1}H-NMR (DMSO-d_6) **12**:2.7[s, 3H, $COCH_3$], 4.3 [s, 2H, CH_2], 6.9 – 8.0 [m, 8H, Ar-H] , 8.2[s, 1H, NH], 10.9[s, 1H, SO_2NH].

Ethyl-9-oxo-1-benzenesulfonamide-1,9-dihydro-[1,2,4]triazolo[5,1-b]quinazoline-2-carboxylate (17).

A mixture of **4** (0.01 mol) and diethyloxalate (0.01 mol) in methanol (20 ml) containing sodium methoxide 0.01M) was refluxed for 8 hr. After cooling, the reaction mixture was poured onto ice water and acidified with dil HCl.

The solid obtained was recrystallized from ethanol to give **17**. Physicochemical and analytical data are listed in table 1. IR (KBr, cm^{-1}) **17**: 3322(NH), 3070(CH-arom.), 2993, 2850(CH-aliph.), 1733, 1650, 1640(3C=O), 1315, 1161(SO_2). MS(m/z) **17**: 413(M^+-$COCH_3$, 0.3%), 318(66.5%), 315(74.12%), 200(73.65%), 172(100%), 155(89%), 108(22%), 89(6%), 65(100%).^{1}H-NMR (DMSO-d_6) **17**:1.3[t, 3H,CH_3], 2.9(s, 3H, $COCH_3$), 4.3 [q, 2H, CH_2], 6.9– 8.0 [m, 8H, Ar-H], 10.9[s, 1H, SO_2NH].

Biological testing

Animals

Male albino mice weighing around 18-20 gms and male Wistar rats weight around 180-200 gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were acclimatized to animal house conditions. Animals were provided with standard diet and water adlibtum. Animals were kept under constant environmental condition and observed daily throughout the experimental work.

Anti-inflammatory activity.

The anti-inflammatory activity was carried out following the method of *Domenjoz, R* [14]. Rats (180 – 200 gm.) were divided into 5 different groups each of 6 animals. At the beginning the thickness of the left paw was measured. They were treated orally with the tested compounds, at 30 mg/ kg body weight or indomethacin 600 mg/kg as a reference standard. After 30 minutes of administration, the inflammation was induced by S.C. injection of 0.1 ml of 6 % formalin solution in normal saline. The right hind paw was injected with an equal volume of saline.

The difference in thickness between the two paws gave the swelling induced by formalin. The anti-inflammatory efficacy was estimated by comparing the swelling of the treated with the control. The difference in thickness was recorded after 30, 60, 90 and 120 minutes.

Determination of LD_{50} of compounds 8, 10 and 17.

Preliminary experiments were carried out on 6 main groups (10 mice/each dose/each group). Compounds **8**, **10** and **17** were injected in different doses to find out the range of doses which cause zero and 100 % mortality of animals. A range doses was determined for each compound.

In group of 10 animals each, compound **8** was given i.p. in doses of 120, 220, 270, 370, 470 and 570 mg/100g b.w. Also, LD_{50} was determined by i.p. injection of compound **10** in different doses 120, 160, 240, 300, 460 and 520 mg/100g b.w. In group of 10 animals each, compound **17** was given i.p. in doses of 150, 200, 250, 300, 350 and 400 mg/100g b.w.

The LD_{50} was evaluated by *Spearman and Karber method* [15] on groups of mice, each of 10 animals. The test compounds were administrated i.p. at different doses. The number of animals which died within 24 h was recorded.

The LD_{50} was then calculated by the application of the following formula:

$$LD_{50} = D_m - \frac{\sum (Z.d)}{n}$$

.D_m = The dose by which killed all the mice in the group
.Z = Half the sum of the dead rats from 2 successive groups
.d = The difference between 2 successive doses
.n = number of animals in each group

Biochemical studies of anti-inflammatory compounds, 8, 10 and 17 on liver enzymes in rats

Experimental Design

This experiment was carried out to examine the effect of anti-inflammatory compounds, **8**, **10** and **17** on liver enzymes. A solution of 6g % for each anti-inflammatory compounds, **8**, **10** and **17** in dimethyl sulfoxide (DMSO) was prepared for intragastric intubation of rats. Groups of animals each consisting of 6 rats in each were treated daily for 10 days as follows:

Group I: Control (was given similar volume of saline p.o.)
Group II: Normal (was given similar volume of DMSO p.o.)
Group III: Was treated with compound **8** (300 mg/kg b.w.) dissolved in DMSO orally in a single daily dose [16].
Group IV: Was treated with compound **10** (300 mg/kg b.w.) dissolved in DMSO orally in a single daily dose [16].
Group V: Was treated with compound **17** (300 mg/kg b.w.) dissolved in DMSO orally in a single daily dose [16].
Group VI: Was treated with indomethacin (600 mg/kg b.w.) dissolved in DMSO orally in a single daily dose [16].
After 10 days of treatment, animals were killed by cervical dislocation, blood samples were withdrawn from the retro-orbital vein of each animal. The separated blood was used for the estimation of SGOT, SGPT, γ-GT, ALP, LDH, TBARS, GSH, GPx and SOD.

Biochemical Assays:

Serum levels of glutamic-oxaloacetic transaminase (GOT) [17], glutamic-pyruvate transaminase (GPT) [17], alkaline phosphatase (ALP) [18], Gamma Glutamyl transferase γ-GT [19], lactate dehydrogenase [20] and TBARS in serum [21]. Blood superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were carried out by Paglia and Valentine [22] and Marklund and Marklund [23], respectively. Blood haemoglobin was determined according to the method of Van Kampen and Zijlstra, [24].

Statistical analysis.

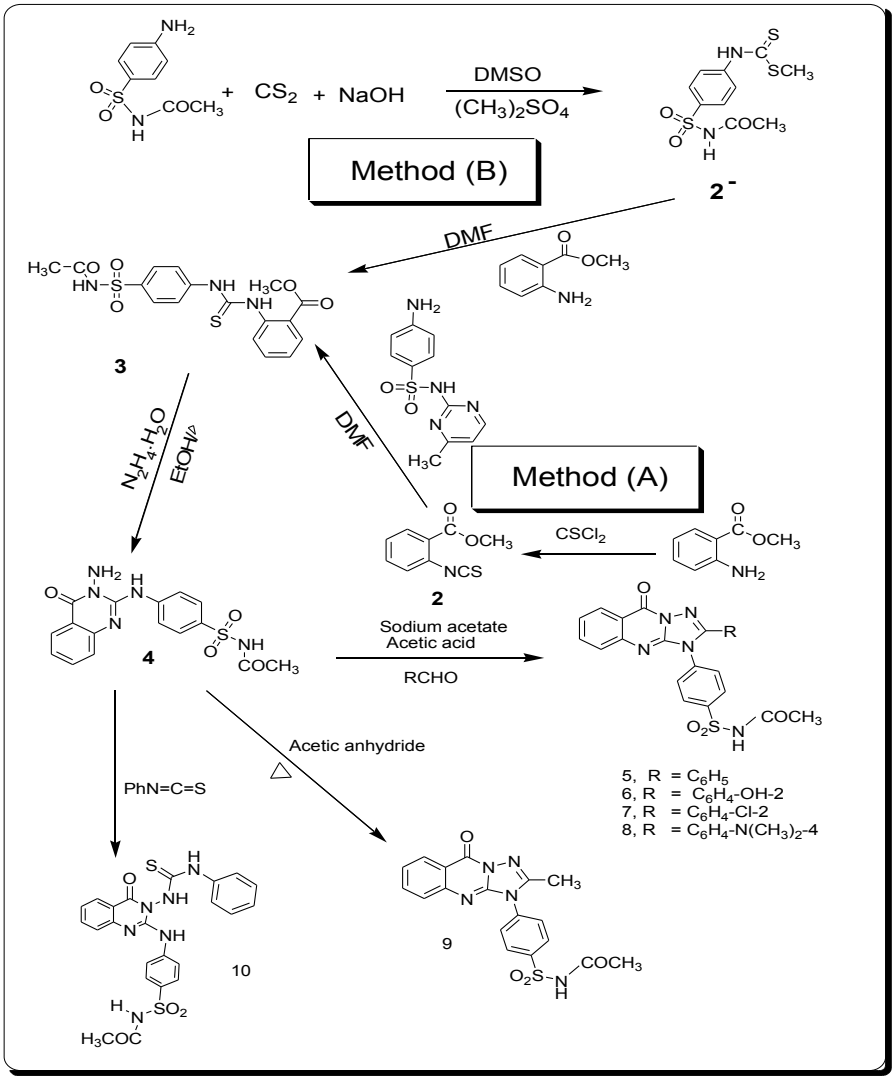
All the grouped data were statistically evaluated with SPSS/7.5 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for ten animals in each group

RESULTS AND DISCUSSION

Chemistry

The starting material methyl-2-isothiocyanatobenzoic acid **2** was synthesized from the reaction of methylanthranilate with thiophosgene. The reactivity of isothiocyanato derivative **2** towards nitrogen nucleophile was investigated herein. When compound **2** was reacted with sulfacetamide in dimethylformamide at room temperature, the thioureido derivative was obtained in good yield **3**. Anther method was designed to synthesis thioureido derivative **3** by adding carbondisulphide and sodium hydroxide solution simultaneously to sulfacetamide in dimethylsulphoxide. Dimethyl sulphate was added to the reaction mixture to get a semi solid methyl [4-(acetylsulfamoyl) phenyl] dithiocarbamate **2'** [25]. The compound **2'** and methyl anthranilate were stirred in dimethylformamide under room temperature for 3-4h furnished the thioureido derivative **3** in high yield Scheme (1). Structure of compound **3** was established based on both elemental and spectral data. The structure of compound **3** was supported by elemental analyses, IR, ^{1}H-NMR and mass spectral data. IR spectrum of compound **3** exhibited the disappearance of NCS band and presence of bands at 3471, 3317(NH), 1701, 1678(2C=O), 1375, 1175 (SO_2), 1299(C=S). ^{1}H-NMR (DMSO-d_6) spectrum of **3** revealed signals at 2.4 [s, 3H, $COCH_3$], 3.6 [s, 3H, OCH_3], 7.3 – 8.4 [m, 10 H, Ar-H + 2 NH], 13.06 [s, 1H, SO_2NH].Treatment of compounds **3** with hydrazine hydrate in ethanol afforded the corresponding N-amino derivative **4** scheme (1). The formation of compound **4** via the reaction of **3** with hydrazine hydrate was assumed to be *via* elimination of 1 mol H_2S followed by intramolecular cyclization [26] to give the N- amino derivative 4 (lead acetate paper). IR spectrum of compound **4** showed bands at 3460, 3460, 3348, 3251(NH, NH_2), 1687, 1650 (2C=O).

Scheme (1):



The mass spectrum of compound **4** showed a molecular ion peak at 377 (M^++ 4, 59.8%) with a base peak at 304(100%). Also, the reaction of **4** with aromatic aldehydes in acetic acid in the presence of fused sodium acetate afforded tricyclic triazoloquinazoline derivatives **5**–**8**, respectively scheme (1). The structure of compounds **5** -**8** was confirmed on the bases of elemental analyses and spectral data. IR spectra of compounds **5** – **8** revealed the absence of NH_2 bands. Mass spectrum of compound **5** revealed a molecular ion peak m/z at 431(M^+-CO, 12.50%), with a base peak at 194 (100 %). Mass spectrum of compound **6** exhibited a molecular ion peak m/z at 458 (M^+-OH, 5.20 %), with a base peak at 287 (100 %). Mass spectrum of compound **7** showed a molecular ion peak m/z at 493 (M^+, 1.76 %), with a base peak at 139 (100 %).^{1}H-NMR spectrum of compound **8** in (DMSO-d_6) exhibited signals at 2.3(s, 6H, $2CH_3$), 2.9(s, 3H, $COCH_3$), 7.0-7.8(m, 13H, Ar-H+NH), 10.7[s, 1H, SO_2NH_2].

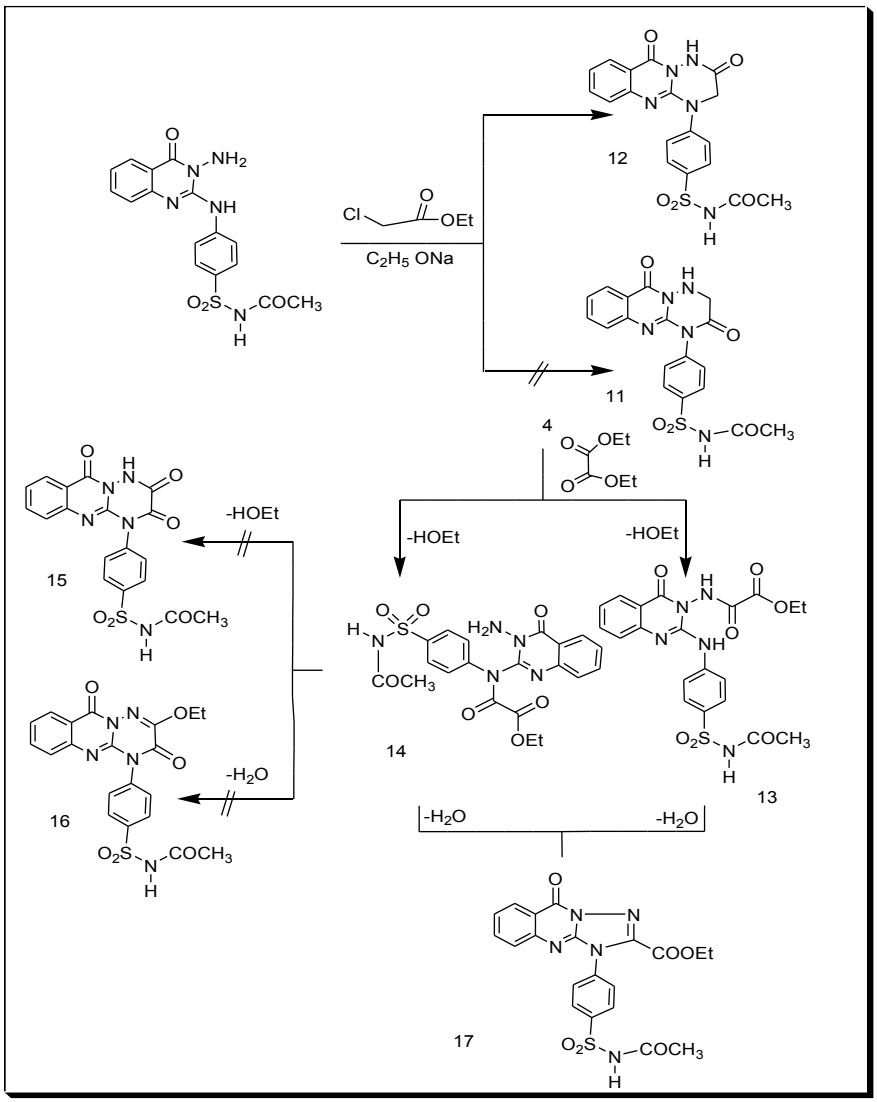
N-Acetyl-4-(2-methyl-9-oxo-9H-[1,2,4]triazolo[5,1-b]quinazolin-3-yl)-ben-zenesulfonamide **9** was obtained *via* reaction of **4** with acetic anhydride; its mass spectrum revealed a molecular ion peak m/z at 382(M^+-CH_3,7.8%), with a base peak at 341(100%).

On the other hand, the reaction of **4** with phenyl isothiocyanate in refluxing ethanol furnished the correspondingN-acetyl-4-[4-oxo-3-(3-phenylthioureido)-3,4-dihydro-quinazoline-2-yl-amino] benzenesulfonamide **10**.

The structure of **10** was established by elemental analysis, IR and mass spectroscopy. IR spectrum of compound **10** showed bands at 3382, 3290(2NH), 1670, 1586(2C=O), 1542(C=N), 1316, 1130(SO_2)., its mass spectrum revealed a molecular ion peak m/z at 508 (M^+, 1.8 %) with a base peak at 287 (100 %).

Finally, the reaction of derivative **4** with ethyl chloroacetate in refluxing sodium methoxide solution yielded the triazinoquinazoline **12** rather than its isomeric structure **11** (Scheme 2).

Scheme (2):



Structure **12** was suggested rather than structure **11**, based on assumption that the reaction basic condition allowed it to proceed through formation of sodium salt on the less basic NH, and elimination of sodium chloride followed by cyclisation [27]. In addition the IR spectrum of isolated product showed a (3C=O) bonds at 1690, 1662, 1624cm^{-1}, which was at less frequency than that expected for structure **11**. Further evidence was the ^{1}H-NMR spectrum which showed a singlet at 4.3 ppm for the methylene protons. its mass spectrum showed a molecular ion peak m/z at 413(M^{+}, 2.1%) with a base peak at 199(100%). Interaction of compound **4** with diethyloxalate gave a triazoloquinazoline derivative **17** scheme (2). This was confirmed by its elemental analysis, H^{1}-NMR mass spectral data. These results are in agreement with the method previously reported [28].^{1}H-NMR (DMSO-d_6) **17**:1.3[t, 3H, CH_3], 4.3 [q, 2H, CH_2]. Mass spectrum of **17** revealed a molecular ion peak m/z at 413(M^{+}-$COCH_3$, 0.3%), with a base peak at 172 (100%).

Biological activity

It is well known from literature that both quinazolines and sulfonamides exhibit a wide range of biological activities. So, it was of interest to design new compounds containing both these biologically active moieties and to study their anti-inflammatory activities.

Anti-inflammatory activity.

This project involved the design and synthesis of various newly synthesized triazoloquinazoline and triazinoquinazoline derivatives containing sulfacetamide moiety to discover structures with high potency against inflammation. The compound **3** was considered as lead molecule for carrying out structure activity relationship studies. The experiments were planned by modifying the functional groups of the compound **3** to find out enhancement or retardation of the said activities. The aim of chemical modification is to enhance the compound **3** anti-inflammatory potency. Three general types of modification of compound **3** are useful [29].

1. Reaction of the compound **3** with hydrazine hydrate to give compound **4** and increases the polarity of the molecule, makes it more anti-inflammatory potency. Compounds containing N atom/atoms are known to be biologically active probably because of less electronegative nature of the N atom due to which electron cloud is easily available to bind the receptor. At the same time, the size of an atom/group of atoms binding to the receptor is very important.

2. Alkylation of compound **9** at the 3 position also allows androgens to be effective because the alkylated derivatives are slowly catabolized by the liver. The methyl group is not removed metabolically and hence the alkylated derivatives mediate the action of the hormone within cells.

3. Other alterations of the compound **4** can be carried out by generation a series of shiff's bases to yield compounds **5**-**8**. Anti-inflammatory potency decreased in order of compounds **7**, **6**, **8** and **5**. The presence of Cl atom, a good withdrawing group in compound **7** makes it more anti-inflammatory potency than compounds **6**, **8** and **5**.

From the data (Table 2), 4-ketonic compounds, i.e. **4-10,** are more active towards inflammation. But the presence of ketone and acetate groups at positions 9 and 2, respectively in compound **17** makes it less anti-inflammatory potency than compounds **4-10**. Also, the presence of 2 ketone groups at positions 3 and 10 in compound **12** makes it more anti-inflammatory potency than compounds **4-10**. This indicates that in these function groups are playing the important role as compound **12** is more active than compound **17** and compound **17** is less active than compound**s 4-10**. Similarly, a decreasing trend of activity is observed for the compounds **5** and **9** (in this order) which contain a phenyl and methyl groups at C-2 position, respectively. The activity is reduced when phenyl in compound **5** is converted to its acetate in compound **17**. This is due to less availability of electron cloud on ethereal oxygen atom which is attracted by the neighbouring ethyl group. This results in less binding to the receptor due to the large size of the acetyl group.

Table 2: Anti-inflammatory activity of the biologically active compounds

		Formalin induced rat paw oedema thickness (mm)/min			
Compd.	Dose in mg/kg b.w	30	60	90	120
Control	--------	9.30±0.030	9.40±0.05	9.20±0.03	9.60±0.03
4	30	8.3±0.08*	8.1±0.06*	7.7±0.04*	7.2±0.05*
5	30	7.9±0.09**	7.7±0.04**	7.1±0.07**	6.96±0.06**
6	30	8.4±0.03**	8.1±0.06**	7.6±0.06**	7.2±0.04**
7	30	6.9±0.03***	6.5±0.06***	5.96±0.07***	5.6±0.08***
8	30	7.8±0.08*	7.6±0.06*	7.2±0.07*	6.8±0.04**
9	30	6.93±0.04**	6.8±0.06**	6.6±0.05**	6.42±0.03**
10	30	6.6±0.05**	6.4±0.03**	6.3±0.07**	6.2±0.04**
12	30	6.5±0.07***	6.1±0.03***	5.76±0.04***	5.5±0.03***
17	30	6.40±0.035**	6.2±0.064**	6.10±0.08**	5.8±0.06**
Indomethacin#	60	6.5±0.08**	6.20±0.07*	6.10±0.09*	5.90±0.08**

Indomethacin is used as a reference#
Significant at P< 0.05*
Significant at P< 0.01**
***Significant at P< 0.005

Determination of LD_{50} of compounds 8, 10 and 17 in adult mice

The results are given in table (3) shows that i.p. injection of compound **8** in doses of 120, 220, 270, 370, 470 and 570 mg/100g b.w. resulted in mortalities of 0, 1, 2, 4, 8 and 10 respectively. The dose of compound **8** that killed half of the mice (LD_{50}) was 370 mg/100g b.w. The results are given in table (4) shows that i.p. injection of compound **10** in doses of 120, 160, 240, 300, 460 and 520 mg/100g b.w. resulted in mortalities of 0, 1, 2, 3, 9 and 10 respectively. The dose of compound **10** that killed half of the mice (LD_{50}) was 338 mg/100g b.w. The results are given in table (5) shows that i.p. injection of compound **17** in doses of 150, 200, 250, 300, 350 and 400 mg/100g b.w. resulted in mortalities of 0, 1, 1, 5, 8 and 10 respectively. The dose of compound **17** that killed half of the mice (LD_{50}) was 295 mg/ 100g b.w.

Table 3: Determination of LD_{50} of compound (8) given i.p. in adult mice

Group Number	Dose (mg/kg)	No. of animals/group	No. of dead animals	(Z)	(d)	(Z.d)
1	170	10	0	0.5	50	25
2	220	10	1	1.5	50	75
3	270	10	2	4	100	400
4	370	10	4	6	100	600
5	470	10	8	9.0	100	900
6	570	10	10	0	00	00

$$LD_{50} = D_m - \frac{\sum (Z.d)}{n}$$

$$LD_{50} = 570 - \frac{2000}{10} = 370 \text{ mg/100g b.w.}$$

Table 4: Determination of LD_{50} of compound (10) given i.p. in adult mice

Group Number	Dose (mg/kg)	No. of animals/group	No. of dead animals	(Z)	(d)	(Z.d)
1	120	10	0	0.5	40	20
2	160	10	1	1.5	80	120
3	240	10	2	2.5	60	150
4	300	10	3	6.0	160	960
5	460	10	9	9.5	60	570
6	520	10	10	0	00	00

$$LD_{50} = D_m - \frac{\sum (Z.d)}{n}$$

$$LD_{50} = 520 - \frac{1820}{10} = 338 \text{ mg/100g b.w}$$

Table 5 Determination of LD_{50} of compound (17) given i.p. in adult mice

Group Number	Dose (mg/kg)	No. of animals/group	No. of dead animals	(Z)	(d)	(Z.d)
1	150	10	0	0.5	50	25
2	200	10	1	1.0	50	50
3	250	10	1	3.5	50	175
4	300	10	5	7.0	50	350
5	350	10	8	9.0	50	450
6	400	10	10	0	00	00

$$LD_{50} = D_m - \frac{\sum (Z.d)}{n}$$

$$LD_{50} = 400 - \frac{1050}{10} = 295 \text{ mg/100 g b.w.}$$

Toxic symptoms: Compounds **8**, **10** and **17** injected mice exhibited an increase in heart rate, rapid respiration with in 1 to 2 hours. There is a general depression in activity with tremors in hind limbs. The mucous of the eye become brownish in colour and the skin and toes bluish. The temperature of the animals extremities dropped with the toes and tail being cool.

Biochemical studies of anti-inflammatory compounds 8, 10 and 17

Administration of compounds **8**, **10** and **17** orally to the rats at dose of 300 mg/kg.b.w. for 10 days showed non-significant changes in liver enzymes SGOT, SGPT, ALP, LDH, γ –GT and serum TBARs as compared with the control group (Table 6). On the other hand, oral administration of indomethacin showed significant increase of serum SGOT, SGPT, ALP, LDH, γ –GT and TBARs as compared with the control group**.**

Table 7 shows the concentration of GSH, SOD and GPx in blood of normal and experimental groups of rats. The levels of GSH, SOD and GPx in treated rats were non-significantly changes as compared with the control group, but administration of indomethacin orally at dose of 600 mg/kg.b.w. showed significant decrease of blood SOD, GPx and GSH as compared with the control group.

Table 6: Levels of glutamic-oxaloacetic transaminase(GOT), glutamic-pyruvate transaminase(GPT), alkaline phosphatase (ALP), Gamma Glutamyl transferase (γ-GT), lactate dehydrogenase (LDH) and lipid peroxides (TBARS) in serum of normal and experimental groups of rats.

Group	SGOT U/l	SGPT U/l	ALP U/l	γ –GT U/l	LDH U/l	TBARs nmol/ml
Normal (Saline)	9.32 1.82 ±	29.65 ± 4.62	29.70 ± 2.95	4.26 1.05 ±	210.43 10.17 ±	1.04 ± 4.18
Control (DMSO)	9.74 1.73 ±	35.82 ± 5.26	31.63 ± 3.73	4.43 ±0.31	217.84 13.36 ±	0.86 ± 4.35
Comp. 8 (mg/kg.b.w 300)	9.75 1.96 ±	27.44 ± 6.3	28.14 ± 5.82	3.98 0.15 ±	195.74 13.5 ±	0.0.9 ± 4.00
Comp. 10 (mg/kg.b.w 300)	9.11 1.55 ±	25.63 ± 7.11	30.5 ± 5.32	4.21 0.07 ±	200.0 16.4 ±	3.95 0.021 ±
Comp. 17 (mg/kg.b.w 300)	8.5 1.95 ±	27.22 ± 4.25	26.43 ± 6.11	3.70 0.45 ±	185.5 11.83 ±	3.8 0.33 ±
Indomethacin (mg/kg.b.w 600)	24.51 *2.08 ±	65.37 ± 6.41*	76.41 ± 5.72*	9.45 *2.11 ±	326.80 *27.44 ±	7.45 *1.79 ±

Compounds 8, 10, 17 and indomethacin were given orally as a single daily dose for 10 days. Control group was compared to normal group. Experimental groups were compared to control group. Values are given as mean ± SD for groups of six animals each. * Significantly different from control group at $p< 0.05$.

Table 7: Level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood of normal and experimental groups of rats.

Group	(% GSH (mg	(SOD (U/g Hb	(GPx (U/g Hb
(Normal (Saline	4.36 ± 62.78	1.97 13.58±	8.76 182.72±
(Control (DMSO	5.10 ± 59.57	2.08 ± 12.70	10.45 ± 179.16
(Comp. 8 (300 mg/kg.b.w	4.22 ± 55.32	1.80 ± 11.95	21.3 ± 190.45
(Comp. 10 (300 mg/kg.b.w	5.7 ± 57.64	2.1 ± 13.00	12.4 ± 175.20
(Comp. 17 (300 mg/kg.b.w	11.3 ± 60.11	3.2 ± 14.25	17.9 ± 180.37
(Indomethacin (600 mg/kg.b.w	*4.56 ± 45.32	*1.89 ± 7.45	*17.04 ± 133.81

Compounds 8, 10, 17 and indomethacin were given orally as a single daily dose for 10 days. Control group was compared to normal group. Experimental groups were compared to control group. Values are given as mean ± SD .for groups of six animals each. * Significantly different from control group at p< 0.05

Conclusion.

A series of triazoloquinazoline and triazoloquinazoline derivatives were synthesized. Also, Compounds **4-17** exhibited promising anti-inflammatory activity, high LD_{50} value and more safe on liver enzymes. Further studies are in progress to study the in vivo effect of Compounds **8**, **10,** and **17** on different blood parameters to ensure its safety and clear its anti-inflammatory mechanism.

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