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# DESIGN AND SYNTHESIS OF ANTI-INFLAMMATORY POTENTIAL OF NOVEL QUINAZOLINE-4(3H)-ONES LINKED THIAZENES THROUGH AMIDE LINKAGE: DOCKING STUDIES

**JAGADEESH BABU VADLAKONDA[*], M. SRINIVASA MURTHY[a] and M. SARITHA[b]**

Department of Pharmaceutical Chemistry, Pulla Reddy Institute of Pharmacy,
Annaram, Medak (Telangana) INDIA
[a]Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical Sciences,
Deshmukhi, HYDERABAD (Telangana) INDIA
[b]Department of Chemistry, Malla Reddy Engineering College for Women, Dulapally,
HYDERABAD (Telangana) INDIA

## ABSTRACT

In an effort to develop an anti-inflammatory agent, we have designed and synthesized a new scaffold by linking quinazolinones with thiazine through an amide linkage. The synthesized compounds were characterized by $^{1}$H NMR, $^{13}$C NMR FTIR and mass spectral data and tested for in-vivo anti-inflammatory activity using Carrageenan induced paw inflammatory model as well as docking studies were performed in order to elucidate structural insights for the anti-inflammatory activity. The investigation of anti-inflammatory activity screening and docking results suggested that the compound QTA-a showed good anti-inflammatory activity among the tested compounds.

**Key words**: Quinazolinone, Thiazine, Amide linkage, Anti-inflammatory activity, Docking studies, etc.

## INTRODUCTION

Inflammatory diseases are becoming most common phenomenon in aging society throughout the world that is linked to various diseases including cardiovascular diseases, cancer and various inflammatory disorders including rheumatoid arthritis, inflammatory bowel diseases, osteo-arthritis, psoriasis, endotoximia and there are several tissue factors that are known to be involved in the pathogenesis of inflammatory reactions such as histamins, bradykinin, inflammatory cytokines, interelukins-6(IL-6) and tumor necrosis

[*]Author for correspondence; E-mail: vjagadi@yahoo.co.in; Ph.: +91 9618953793

alpha (TNF-α, secretory phospholipase $A_2$, cyclooxygenase and soyabean oxygenase. The inhibition of (TNF-α, inflammatory cytokines, over expression of cytokines and cyclooxygenase has been recognised as an attractive molecular target for design and development of novel anti-inflammatory agents.[1]

Many quinazoline derivatives are known to possess diverse pharmacological activities like anti-microbial and anti-inflammatory, anti-hypnotic,[2] anti-fungal[3], antioxidant[4], anti-cancer[5,6] and anti-convulsant activity[7] and so. Hence quinazoline ester can be considered as useful tool to synthesize many novel compounds through amide linkage with amine containing thiazine derivatives. For synthesized compounds acute toxicity studies were performed according to OECD guidelines. Determination of anti-inflammatory activity was done by Carrageenan induced rat paw oedema model.

Molecular docking studies were performed by using GLIDE XP module of Schrodinger suite for the selected quinazoline derivatives which were screened for in-vitro COX-2 inhibition.

## EXPERIMENTAL

### Materials and methods

### Chemistry

All chemical were purchased from commercial sources. The melting point of all the compounds were determined by open capillary and are uncorrected/unchanged. The purity test was done by TLC method. IR spectra were recorded in KBr on Shimadzu FT – IR 8300 spectrophotometer[1]. H NMR spectra were recorded on Varian 400 MHz spectrometer using DMSO as solvent and tetra methyl silane as an internal standard. Mass spectra were recorded on Aglient 6430 Triple Quadruple LC-MS system.

### Synthesis of ethyl-4-oxo-3H-quinazoline-2-carboxylate[8]

A mixture of anthranilamide (50 g) and diethyl oxalate (100 mL) was refluxed in oil bath at 185-186$^{o}$C for 4.5 hrs. The reaction mixture exhibited a yellowish green fluorescence. Excess of diethyl oxalate was removed by distillation under reduced pressure. The brownish residue was triturated with cold ethanol, filtered, dried and recrystallized from ethanol to give 30 g (86.47%), mp.: 193-195$^{o}$C, lit.189$^{o}$C.[9]

### Synthesis of chalcones [I-III][9]

Quantities of anisaldehyde/3-chlorobenzaldehyde/3-nitro benzaldehyde (0.01 mol) and acetophenone (0.01 mol) were dissolved in minimum amount of alcohol. Sodium

hydroxide solution (0.02 mol) was added slowly and the mixture stirred for 2 hrs until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400 mL of water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and recrystallized from ethanol. Finally, the compounds synthesized were, 3-(4-methoxyphenyl)-1phenylprop-2-en-1-one (I), 3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (II), and 3(3-nitrophenyl)-1-phenylprop-2-en-1-one (III), respectively. The completion of the reaction was monitored by TLC.

**Preparation of thiazine derivatives [I-III a, I-III b]**[10]

A mixture of Chalcone I, II, III (0.02 mol), thiourea/urea (0.02 mol) were dissolved in ethanolic sodium hydroxide (10 mL) was stirred about 2-3 hours with a magnetic stirrer. This was then transferred into 400 mL of cold water with continuous stirring for an hour and then kept in refrigerator for 24 hours for further precipitation. The precipitate obtained was filtered, washed and recrystallized. The completion of the reaction was monitored by TLC.

**Synthesis of quinazoline-4(3h)-ones linked thiazine derivatives**[11]

Synthesis of ethyl-4-oxo-3H-quinazoline-2-carboxylate (0.01 mol) and corresponding amines containing thiazine (0.01 mole) are taken in a round bottom flask then glacial acetic acid was added slowly while shaking. The mixture was heated under reflux for 4-6 hrs. After cooling, the contents were poured into crushed ice. The resulting solid was washed with distill water, filtered, dried in vaccum and recrytallized from warm ethanol.

**N-(6-(4-(dimethylamino) phenyl)-4-phenyl-6H-1, 3-thiazin-2-yl)-4-oxo-3, 4-dihyro-qunazoline-3-carboxamide (QTA-1)**

TLC solventsystem: n-Hexane: Ethyl acetate (3:2), Rf value: 0.72. IR (KBr, $cm^{-1}$): 1637 (NH-CO-), 2369 (C-S-C str), 1497 (Ar C=C), 1385 (Ar-C-O), 1674 (NH-Quinazoline), 1600 (C=N), 3080 (N-H). $^{1}$H-NMR (DMSO-$d_6$400 MHz, δ ppm): 6.94-7.94 (m, $^{14}$H, Ar-H), 4.42(d, CH), 8.0 (NH-Quinazolin and -NH-CO- amide linkage). $^{13}$C NMR: 165.4, 161.0, 160.8, 158.0, 149.5, 145.5, 141.3, 141.1, 133.4, 129.4, 128.6, 127.3, 126.4, 126.7, 126.6, 120.8, 112.9, 112.4, 41.3. Mass spectrophotometry (m/z): 482.16 (M+1).

**N-(4, 6-diphenyl-6H-1, 3-thiazin-2-yl)-4-oxo-3, 4-dihyroqunazoline-3-carboxamide (QTA-6)**

TLC solvent system: n-Hexane:Ethyl acetate (3:2), Rf value: 0.78. IR (KBr, $cm^{-1}$): 1637 (-NH-CO-), 2369 (C-S-C str), 1497 (Ar C=C), 1385 (Ar-C-O), 1674 (NH-Quinazoline), 1600 (C=N). $^{1}$H NMR (DMSO-$d_6$ 400 MHz, δ ppm): 6.94-7.94 (m, 15H, Ar-H), 4.42 (d, CH), 8.0 (NH-Quinazoline and -NH-CO- amide linkage). $^{13}$C NMR: 165.4, 161.0, 160.8,

158.0, 149.5, 145.5, 141.3, 141.1, 133.4, 129.4, 128.6, 127.3, 126.4, 126.7, 126.6, 120.8, 112.9, 112.4. MS (m/z): 439 (M+1).

**N-(6-(4-(hydroxy)phenyl)-4-phenyl-6H-1, 3-thiazin-2-yl)-4-oxo-3,4-dihyroqunazoline-3-carboxamide (QTA-4)**

TLC solvent system: n-Hexane: Ethyl acetate (3:2), $R_f$ value: 0.81. IR (KBr, $cm^{-1}$): 1637 (-NH-CO-), 2369 (C-S-C str), 1497 (Ar C=C), 1385 (Ar-C-O), 1674 (NH-Quinazoline), 1600 (C=N). $^1$H NMR (DMSO-$d_6$ 400 MHz, δ ppm): 6.94-7.94 (m, 15H, Ar-H), 4.42 (d, CH), 8.0 (NH-Quinazoline and -NH-CO- amide linkage). $^{13}$C NMR: 165.4, 161.0, 160.8, 158.0, 154.8, 149.5, 145.5, 141.3, 141.1, 133.4, 129.4, 128.6, 127.3, 126.4, 126.7, 126.6, 120.8, 112.9, 112.4. MS (m/z): 455.1 (M+1).

**Pharmacological activity**

**Determination of acute toxicity (LD50)**

**Method:** The acute toxicity of synthesized compounds was determined by using albino mice of either sex (20-30 g), maintained under standard husbandry conditions. The animals were fasted overnight prior to the experiment and fixed dose (OECD guideline No. 425) method of CPCSEA was adopted for toxicity studies.[12] Effective dose ED50[13] Therapeutic dose is taken as 1/5$^{th}$ of lethal dose.

**Method for determination of anti-inflammatory activity**

**By Carrageenan induced rat paw oedema model**[14,15]

Six groups of albino rats of either sex (each comprising of six animals) weighing between 160-200 g were deprived of food and water for 18 hours prior to the experiment.

Treatment protocol was done as follows:

Group I : Control (5% tween 80)

Group II : Standard drug (Diclofenac sodium 20 mg/kg. In distilled water p.o)

Group III : QTA-a (100 mg/kg p.o) 5% tween 80 suspension

Group IV : QTA-b (100 mg/kg p.o) 5% tween 80 suspension

Group V : QTA-c (100 mg/kg p.o) 5% tween 80 suspension

Group VI : QTA-d (100 mg/kg p.o) 5% tween 80 suspension

Group VII : QTA-e (100 mg/kg p.o) 5% tween 80 suspension

Group VIII : QTA-f (100 mg/kg p.o) 5% tween 80 suspension

The standard diclofenac sodium and synthesized compounds under study i.e. QTA-a, QTA-b, QTA-c, QTA-d, QTA-e and QTA-f were administered orally to all rats. After 30 min 0.1 mL of 1% carrageenan suspension in normal saline was injected in to the sub plantar region of the hind paw of each rat. The oedema volumes of the injected paws were measured at 1/2, 1$^{st}$, 2$^{nd}$ and 4$^{th}$ hr. The difference between the paw volumes of treated animals were compared with that of the control group and the mean oedema volume was calculated.

From the data obtained mean volume of oedema, and percentage reduction in oedema were calculated. Percentage reduction or inhibition in edema volume was calculated by using the formula.

Percentage reduction in oedema volume was calculated by using the formula,

$$\text{Percentage of edema inhibition} = (Vo - V_1)/Vo * 100$$

Where, Vo = Volume of the paw of control at time 't'

$V_1$ = Volume of the paw of drug treated at time 't'

**Molecular docking**

Molecular docking studies by using GLIDE XP module of Schrodinger suite were performed for the selected quinazoline derivatives, which were screened for *in vitro* COX-2 inhibition. Initially, a digitalized structure of the protein COX-2 was retrieved from the protein data bank with PDB ID: 3NTI. Structure of the protein was processed by adding hydrogen to satisfy the valence and optimized by using OPLS-2005 force field (optimized potential for liquid simulations). Receptor grid generation was accomplished using Glide docking protocol and ligands were docked by employing XP mode of Glide. Best pose of each ligand was ranked according to the E-model energy. The docking score from Glide (Glide Score) is entirely based on Chem Score. It also include a steric-clash term, adds polar terms featured by Schrodinger to correct electrostatic mismatches.

G score = 0.065 x Van der Waals energy + 0.130 x Coulomb energy + Lipophilic tern (Hydrophobic interaction) +H bonding + Metal binding + Bury P (Penalty for buried polar groups) + Rot B (Penalty for freezing rotatable bond) + Site (Polar interactions in the active site).[16]

## RESULTS AND DISCUSSION

In the present, ethyl-4-oxo-3H quinqzoline-2-carboxylate (3) was prepared by reaction between anthranilamide (1) and diethyl oxalate (2) heating at 180-186$^{o}$C. Chalcones

(6a-6f) were prepared by base catalysed claisen-schmidit condensation between different aldehyde and acetophenone further, compounds 6a-6af were treated with Thio urea to give series of thiazine derivatives (7a-7f) followed by interaction of these compounds 7(a-f) with ethyl-4-oxo-3H quinqzoline-2-carboxylate in the presence of acetic acid to give a series of qunazoline linked thiazine derivatives 8(a-f).

Compound 3 was confirmed by melting point 193-194$^{o}$C, lit 189$^{o}$C. chalcone compounds 6(a-f) were confirmed by carbonyl peak around 1647 $cm^{-1}$ and c=c stretching around 1569 $cm^{-1}$ in IR spectra. Further, thiazine compounds were 7(a-f) confirmed by primary amine peak around 1612 $cm^{-1}$ and c-s-s stretching peak around 2369 in IR spectra and δ 2.3 as singlet, -CH proton of thiazine at δ 4.64 in H NMR.

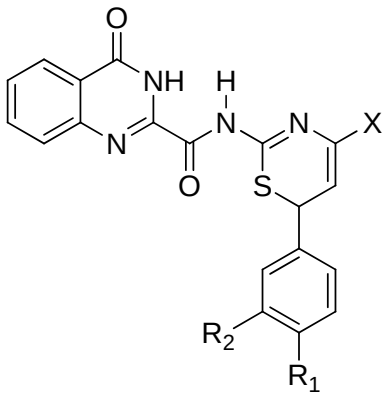
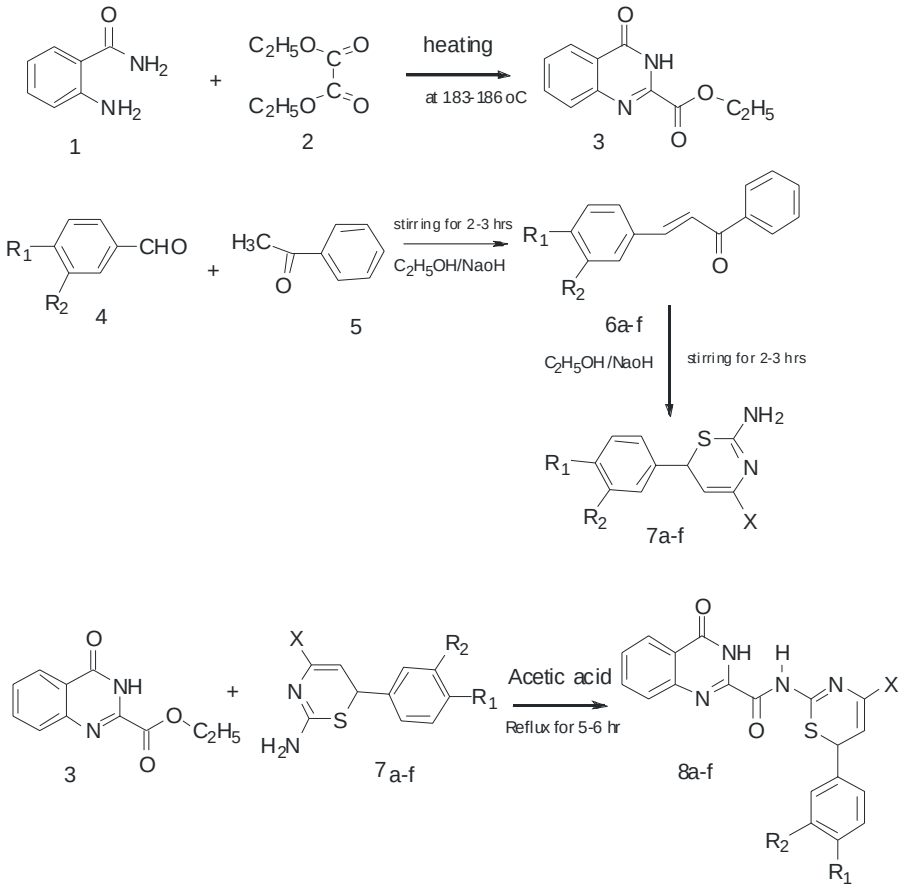



**Fig. 1: General structure of quinazoline linked thiazine derivatives**

**Table 1: Physical characterization data of synthesized compounds**

| Product code | $R_1$ | $R_2$ | X | Molecular formula | Molecular weight | Solvent for recrystalli-zation | M.P. ($^{0}$C) | Yield (%) |
|---|---|---|---|---|---|---|---|---|
| QTA-a | -N($CH_3$)$_2$ | H | $C_6H_5$ | $C_{27}H_{23}N_5O_2S$ | 481.16 | Ethanol | 201-210 | 71 |
| QTA-b | -P-Cl | H | $C_6H_5$ | $C_{25}H_{17}N_4O_2SCl$ | 472.08 | Ethanol | 215-216 | 62 |
| QTA-c | -P-OH | H | $C_6H_5$ | $C_{25}H_{18}N_4O_3S$ | 454.50 | Ethanol | 231-232 | 68 |
| QTA-d | -P-$No_2$ | H | $C_6H_5$ | $C_{25}H_{17}N_5O_4S$ | 483.0 | Ethanol | 241-243 | 70 |
| QTA-e | -P-$OCH_3$ | H | $C_6H_5$ | $C_{26}H_{20}N_4O_3S$ | 468.50 | Ethanol | 206-208 | 72 |
| QTA-f | H | H | $C_6H_5$ | $C_{25}H_{18}N_4O_2S$ | 438 | Ethanol | 219-220 | 67 |

The final compounds quinazoline linked thiazine derivatives 8(a-f) were confirmed by (-NH-CO) -amide peak appear around at 1637 $cm^{-1}$ in proton NMR it was appeared at δ 10.23 as singlet. The quinazoline linked thiazine derivatives were confirmed by FTIR, H NMR, $C^{13}$ NMR and mass spectral data.




| Compound code | $R_1$ | $R_2$ | X |
|---|---|---|---|
| 6a, 7a, 8a | | H | $C_2H_5$ |
| 6b, 7b, 8b | P-Cl | H | $C_2H_5$ |
| 6c, 7c, 8c | P-OH | H | $C_2H_5$ |
| 6d, 7d, 8d | P-$NO_2$ | H | $C_2H_5$ |
| 6e, 7e, 8e | P-$OCH_3$ | H | $C_2H_5$ |
| 6f, 7f, 8f | H | H | $C_2H_5$ |

**Fig. 2: Synthesis of titled compounds 7a-f**

**Table 2: Elemental analysis of synthesized compounds**

| Compound code | Elemental analysis (Calculated) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **% C** | **% H** | **% N** | **% O** | **% S** | **% Cl** | **% NO** |
| QTA-a | 67.34 | 4.81 | 14.54 | 8.64 | 6.66 | - | - |
| QTA-b | 63.49 | 3.62 | 11.85 | 6.77 | 6.77 | 7.50 | - |
| QTA-c | 66.07 | 3.99 | 12.33 | 1056 | 7.05 | - | - |
| QTA-d | 31.43 | 1.79 | 5.86 | 3.35 | 3.36 | - | 54.21 |
| QTA-e | 66.65 | 4.30 | 11.96 | 1024 | 6.84 | - | - |
| QTA-f | 66.48 | 4.41 | 12.78 | 7.30 | 7.31 | - | - |

% C = Carbon percentage, % H = Hydrogen percentage, % N = Nitrogen percentage,
% O = Oxygen percentage, % S = Sulphur percentage, % Cl = Chlorine percentage,
% NO = Nitric oxide percentage

**Table 3: Data showing anti-inflammatory activity of quinazoline linked thiazine derivatives in carrageenan induced acute rat paw oedema model**

| Group | Treatment | Dose mg/kg | Paw oedema volume | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **After 1/2 hr** | | **After 1st hr** | | **After 2nd hr** | | **After 4th hr** | |
| | | | **Mean** | **% ROV** | **Mean** | **% ROV** | **Mean** | **% ROV** | **Mean** | **% ROV** |
| **1** | Control | 0.5 mL | 0.18 | - | 0.51 | - | 0.58 | - | 0.55 | - |
| **2** | Standard | 20 | 0.101 | 45.45 | 0.16 | 67.74 | 0.116 | 80.00 | 0.016 | 96.96 |
| **3** | QTA-1 | 100 | 0.065 | 65.00 | 0.08 | 68.57 | 0.15 | 68.57 | 0.15 | 73.00 |
| **4** | QTA-2 | 100 | 0.13 | 23.33 | 0.30 | 42.85 | 0.40 | 50.00 | 0.30 | 56.94 |
| **5** | QTA-3 | 100 | 0.14 | 24.44 | 0.40 | 43.95 | 0.50 | 60.00 | 0.40 | 57.94 |
| **6** | QTA-4 | 100 | 0.18 | 40.00 | 0.25 | 58.33 | 0.24 | 65.71 | 0.22 | 67.57 |
| **7** | QTA-5 | 100 | 0.18 | 40.00 | 0.27 | 55.00 | 0.30 | 57.14 | 0.35 | 51.00 |
| **8** | QTA-6 | 100 | 0.22 | 25.00 | 0.35 | 35.71 | 0.45 | 40.66 | 0.36 | 45.42 |

ROV- Reduction in paw oedema volume

## Molecular docking

Molecular docking study was performed for further exploration of the mechanism of action of the synthesized compounds with cox-2 enzyme and to elucidate the observed

biological results. Docking of of compound QTA-I showed two hydrogen bond interaction (one from Quinazoline ring -NH and other one from -NH-C=O amide linkage) and Tyr 355; d = 1.81 $A^o$ and 2.41 and quinazoline linked thiazine ring showed stacking interaction with ARG 120.Moreover, compound was surrounded by VAL 523, SER 353, GLY 524, LEU 352 and SER 471, which was very similar to that of the interaction exhibited by the know cox-2 inhibitors. In addition to this, QTA-I compound has dimethyl amino group which provided additional interaction with active site amino acid of cox-2 and this might be contributed to better activity than remaining compounds. Further, this is supported by results obtained from *in vivo* anti-inflammatory acitivity.

The two dimentional and three dimentional represent of compound QTA-a were given below.

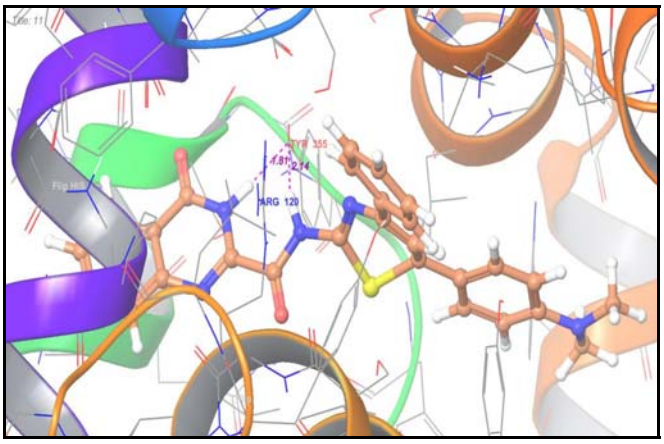



**Fig. 3: Three – dimensional structural model of compound QTA-2 into COX-2**

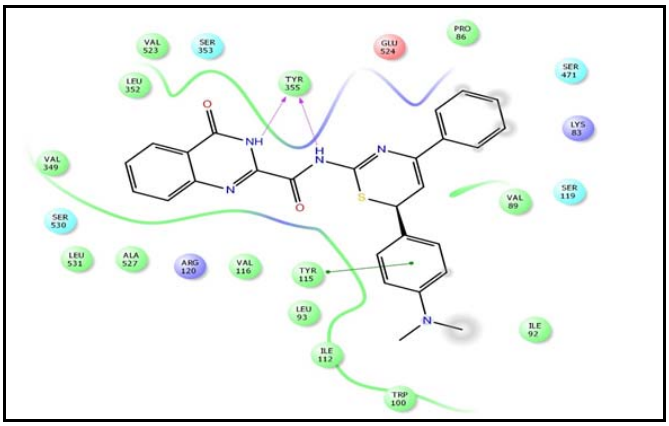



**Fig. 4: Two-dimensional representation of the interacting mode of QTA-a with COX-2**

## CONCLUSION

The synthesizes compounds were evaluated for *in vivo* anti-inflammatory activity. Among the evaluated compounds QTA-aexhibited highest inherent anti-inflammatory activity due to electron donating character of dimethyl amine on phenyl nucleus.

In addition to this, QTA-a compound showed significant docking interaction with cox-2 active site. Based on these observation, ATA-a have proven the potential as valuable lead for anti-inflammatory activity and remaining compounds exhibited mild to moderate activity compared to the standard compound (Diclofenacsodium).

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