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Original article

Synthesis, biological evaluation and molecular docking of novel series of spiro [(2H,3H) quinazoline-2,1′- cyclohexan]-4(1H)- one derivatives as anti-inflammatory and analgesic agents

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ABSTRACT

Three series of Spiro [(2H,3H) quinazoline-2,1′-cyclohexan]-4(1H)-one derivatives have been synthesized. Some of the novel quinazolinone derivatives IIe, VIIIc, XIc, XIIb, XIIc, XVIb showed considerable potent anti-inflammatory and analgesic activity of superior G.I.T. safety profile in experimental rats in comparing to indomethacin and tramadol as reference drugs. Docking study into COX-2 has been made for derivatives of highest anti-inflammatory activity. The compound XVIb showed the nearest RMSD value to that of indomethacin.

1. Introduction

It is well known that non steroidal anti-inflammatory drugs (NSAIDs) are associated with several side effects such as gastrointestinal mucosal damage, bleeding, intolerance and renal toxicity [1,2]. Production of safer and more active NSAIDs and analgesic drugs is still needed. Quinazoline and quinazolinone nuclei have drawn a great attention due to their wide range of chemotherapeutic activities including antiviral [3], antibacterial [4,5], antifungal [6,7], antimalarial [8], anticancer [9–11], antihypertensive [12], diuretic [13,14], inhibitors of derived growth factor receptor phosphorylation [15], anticonvulsant [16], ghrelin receptor antagonists [17], anti-inflammatory, analgesic and COX-II inhibitors [18–20]. Additionally, different known anti-inflammatory drugs such as: **Proquazone**, 1-isopropyl-7-methyl-4-phenylquinazoline-2(1H)-one(**1**) [21], **Fluoroquazone**, 4-(4-fluorophenyl)-7-methyl-1-propan-2-yl-quinazoline-2-one (**2**) [22,23], (**Tryptanthrin**) indolo [2,1-b] quinazoline alkaloid (**3**) [24] are bearing quinazolinone nucleus. Also, it has been reported that substitution pattern by different aryl or heteroaryl moieties at 2/3 position of quinazoline nucleus markedly influences the anti-inflammatory activity [25]. On the other hand, benzene sulfonamides [26], 2-oxo(imino)pyridines [27,28], pyrazoles [29–31], pyrimidines [32], imidazoles [33] and thiazolidinones [34–36] are other important pharmacodynamic heterocyclic nuclei which when incorporated into different heterocyclic templates, have been reported to possess potent anti-inflammatory activity. The enhanced overall lipophilic characteristics of the target compounds could favor their selectivity towards COX-2 enzyme over COX-1 leading to increase of GIT safety margin [26]. Based on the above observations and in continuation of our anti-inflammatory and analgesic drug research program, it was of interest to synthesize a novel series of quinazolinone derivatives with structure modifications involving incorporation of the above mentioned heterocyclic moieties at 3rd position and a spiro cyclohexane moiety at 2nd position of quinazolinone moiety as a trial to obtain safer and potent anti-inflammatory and analgesic agents. The ulcerogenic activity of the active compounds was determined.

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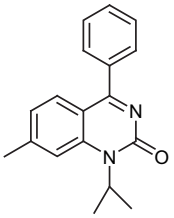

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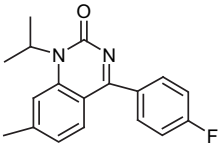

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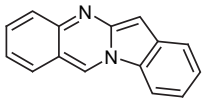

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2. Chemistry

The desired spiro [(2H, 3H)-3(4-substituted aminosulfonyl phenyl) quinazoline-2,1′-cyclohexan]-4(1H) ones (**IIa–e**) were obtained by aromatic aminolysis [37] of the known benzoxazine derivative, namely: spiro [2H-3,1-benzoxazine-2,1′-cyclohexan]-4(1H)-one (**I**) [38,39], upon reaction with the appropriate sulfa drugs, namely: sulfanilamide, sulfaguanidine, sulfamethoxazole, sulfapyridine and sulfadiazine in refluxing glacial acetic acid in the presence of anhydrous sodium acetate. The sulfonyl urea or (thiourea) derivatives **IIIa–c** were prepared in good yields by the reaction of aminosulfonylphenyl derivative **IIa** with ethyl isothiocyanate, cyclohexyl and phenyl isocyanates in refluxing dry acetone in the presence of anhydrous potassium carbonate. N-alkylation of the derivative **IIa** was carried out by its reaction with ethyl iodide in refluxing ethanol in the presence of anhydrous potassium hydroxide to give the corresponding *N*-ethyl-lquinazolinone derivative, namely, spiro [(2H,3H)-3(4-aminosulfonylphenyl)-1-ethylquinazoline-2,1′-cyclohexan]-4(1H)one (**IV**) (Scheme 1).

Also, the starting benzoxazine derivative **I** was allowed to condense with *p*-amino acetophenone in glacial acetic acid in the presence of anhydrous sodium acetate to give the corresponding key intermediate, 3-(*p*-acetyl phenyl)quinazolin-4(1H)one derivative **V**. Claisen–Schmidt condensation of the acetyl derivative **V** with different aldehydes namely, benzaldehyde, *N*,*N* dimethylaminobenzaldehyde and\or furan-2-carboxaldehyde in 5% ethanolic sodium hydroxide solution afforded the corresponding α,β-unsaturated ketones (chalcones) **VIa–c** respectively. These chalcones are considered to be useful intermediates in several cyclization reactions to produce different types of heterocyclic compounds of diverse biological importance, according to the reactants used and the reactions conditions [40,41].

Cyclocondensation of the unsaturated ketones **VIb**,**c** by hydrazine hydrate in absolute ethanol afforded the corresponding pyrazoline derivatives **VIIa**,**b**, but when the reaction was carried out in glacial acetic acid, the N-acetyl pyrazoline derivatives **VIIc**,**d** were obtained.

Also, cyclocondensation of the key ketones **VIb**,**c** by methyl hydrazine and/or phenyl hydrazine in absolute ethanol furnished the target *N*-methyl and/or *N*-phenyl pyrazoline derivatives **VIIIa–d**. Further, when α,β-unsaturated ketones **VIb**,**c** were allowed to react with hydroxylamine hydrochloride in ethanolic sodium hydroxide solution, afforded the corresponding isoxazolines **IXa**,**b**, respectively, while their reaction with thiourea in boiling ethanol in the presence of potassium hydroxide, gave the corresponding pyrimidine-2-thione derivatives **XIa**,**b**, according to reported methods [42].

On the other hand, it is well known that several pyridone and imino pyridine derivatives are of chemotherapeutic importance [43]. In this study, 2(1H) pyridones **XIa–c** were obtained following the reported procedure [43,44] by heating a mixture of equimolar amounts of the acetyl derivative **V** with the appropriate aromatic aldehyde namely: benzaldehyde, p-flourobenzaldehyde and/or 2-hydroxy benzaldehyde and ethyl cyanoacetate in the presence of excess ammonium acetate in n-butanol. Upon applying the same procedure, using malononitrile instead of ethyl cyanoacetate, the target 2(1H) iminopyridines **XIIa–c** were obtained (Scheme 2).

Additionally, as a structure variation of the para substitution of the phenyl ring at the 3-position of the quinazolinone moiety, the ester derivative **XIII** was prepared by condensation of benzoxazine derivative **I** with ethyl-*p*-aminobenzoate in refluxing glacial acetic acid in the presence of anhydrous sodium acetate. The preparation of acid hydrazide **XIV** was achieved through hydrinolysis of the ester precursor **XIII** with excess hydrazine hydrate. Treatment of the derivative **XIV** with *p*-methoxybenzaldehyde and/or 2-thiophenaldehyde in absolute ethanol containing few drops of acetic acid afforded the desired Schiff's base derivatives **XVa**,**b** in moderate yields. Furthermore, the intermediate Schiff bases **XVa**,**b** were cyclized into the corresponding thiazolidine-4-one derivatives **XVIa**,**b** by their cyclocondensation reaction by thioglycolic acid in refluxing dry benzene [45]. Synthesis of unsymmetrical ureas and/or thioureas **XVIIa–c** was achieved via the reaction of the hydrazide derivative **XIV** with cyclohexyl isocyanate and cyclohexyl and/or phenyl isothiocyanate in dry benzene containing few drops of triethylamine. Finally, preparation of the imidazolidinone derivatives **XVIIIa–c** were achieved by the reaction of the derivatives **XVIIa–c** with chloroacetic acid in the presence of few drops of pyridine, while their reaction with the bielectrophilic species, malonic acid, it gave the corresponding, 4,6-dioxo-2-thio(oxo)pyrimidin e.g. derivatives **XIXa–c** in moderate yields. (Scheme 3).

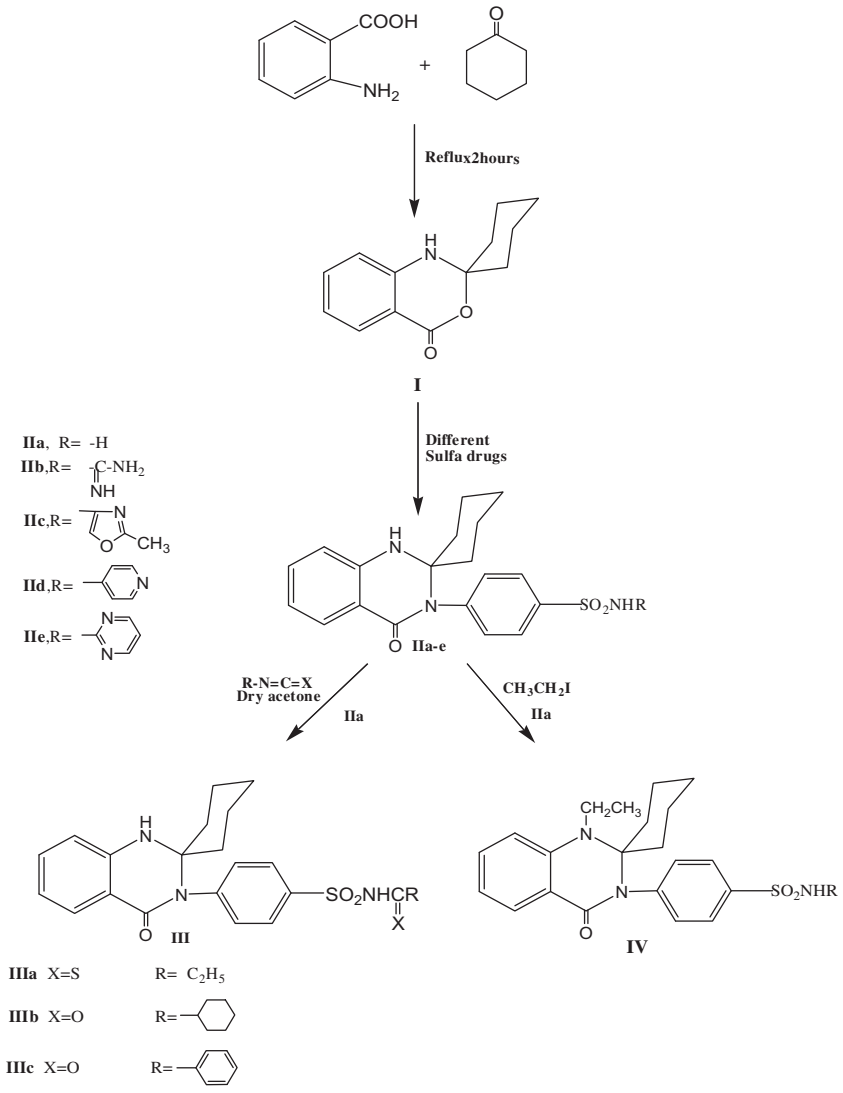
3. Results and discussion

3.1. Biological evaluation

3.1.1. Antiinflammatory effect

The anti-inflammatory activity of nineteen of the newly synthesized compounds: **IIa**, **IIe**, **IIIa**, **V**, **VIc**,**VIId**, **VIIIc**, **VIIId**, **XIb**, **XIc**, **XIIb**, **XIIc** ,**XIII**, **XIV**, **XVa**, **XVIb**, **XVIIb**, **XVIIIb**, **XIXb** were evaluated by applying carrageenan-induced paw oedema bioassay in rats [46] using indomethacin as a reference standard. Results were expressed as mean ± S.E. Difference between vehicle control and treatment groups were tested using one way ANOVA followed by the least significant difference (L.S.D.). Methods of statistical analysis were done according to Armitage et al. [47].

According to Table 1, administration of many of tested compounds 60 min prior to carrageenan injection at dose of 9 mg/kgb wt caused significant inhibition of paw oedema response. Compounds **IIe**, **VIIIc**, **XIIc** and **XVIb** caused significant decrease in paw oedeama after 2, 3, 4 h after drug administration, while **XIc**, **XIII** and **XVIIIb** gave their response after 2 h of administration and continued to the third hour. Compounds **XIb** and **XIIb** showed the effect only after 2 h but compounds **VIId**, **XIV** significantly decreased the paw oedeama after 4 h post administration. On the other hand compounds **IIa**, **IIIa**, **V**, **VIc**, **VIIId**, **XVa**, **XVIIb** and **XIXb** were inactive towards carrageenan-induced oedema in comparison to the standard reference indomethacin which markedly and



Scheme 1.

significantly inhibited the paw oedema after 2, 3, 4 h of carrageenan injection. Thus, compounds **IIe**, **VIId**, **VIIIc**, **XIb**, **XIc**, **XIIb**, **XIIc**, **XIII**, **XIV**, **XVIb** and **XVIIIb** have good anti-inflammatory activity and compound **XVIb** was the most potent derivative. Results are illustrated by Fig. 1.

3.1.2. Analgesic activity

The analgesic activity of the above mentioned nineteen derivatives was also evaluated by applying Hot plate test [48] using tramadol as a standard reference. Results were expressed as mean ± S.E. Difference between vehicle control and treatment groups were tested using one way ANOVA followed by the least significant difference (L.S.D.). Methods of statistical analysis were done according to Armitage et al. [47].

According to Table 2, compounds **IIe**, **IIIa**, **XIc**, **XIIb**, **XVIb**, showed significant analgesic activity higher than that obtained by Tramadol 1 h and 2 h post administration.

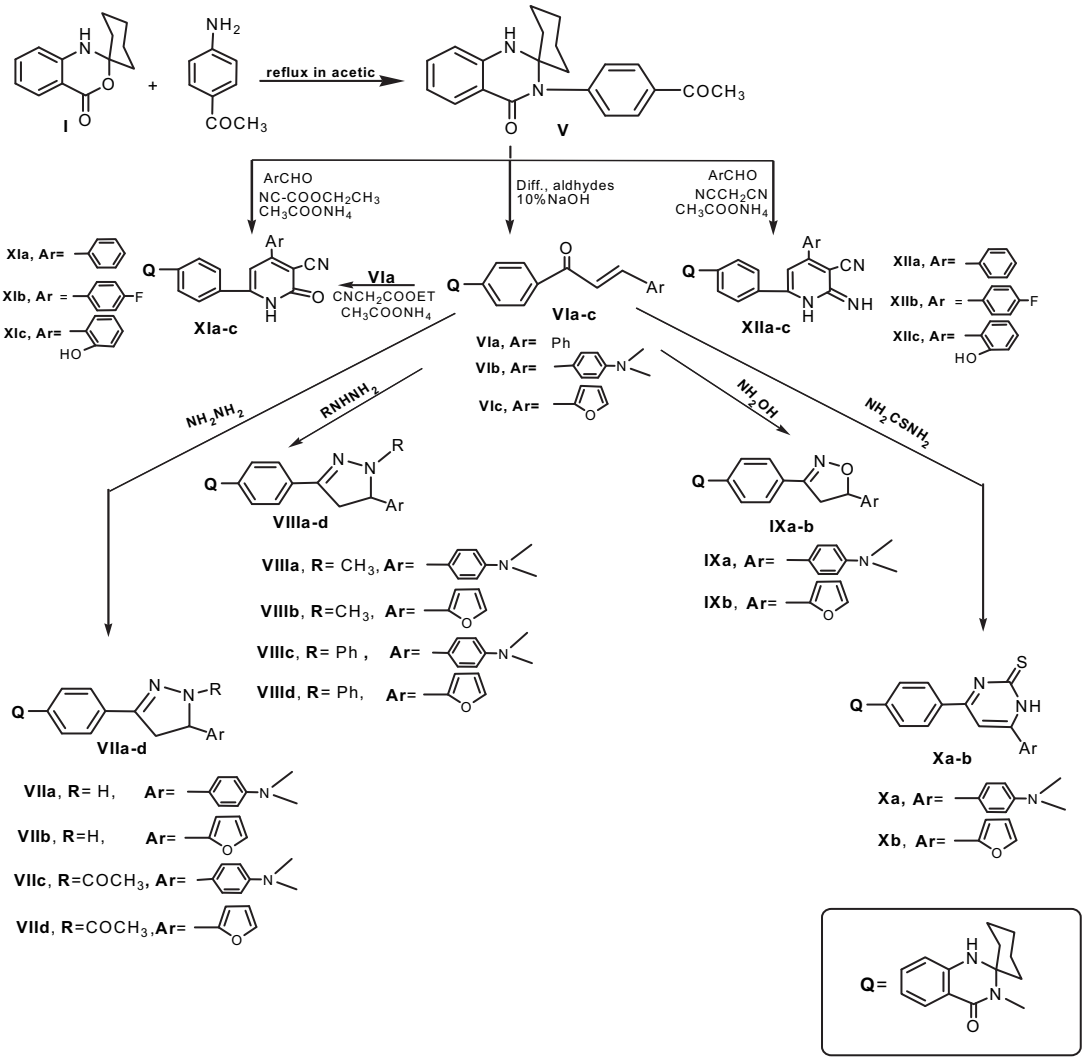
While compounds **IIa**, **V**, **VIc**, **XVIIb**, **XIXb** exhibited equipotent analgesic effect or slightly less than that of Tramadol after 1 and 2 h of their administration. Compounds **VIIIc**, **XIIc** and **XVa** exhibited significant analgesic activity higher than or slightly equipotent to Tramadol only after 2 h of administration. Compounds **VIId** and **XIV** exhibited the analgesic effect after 1 h of administration only. Compounds **VIIId**, **XIb**, **XIII**, **XVIIIb** have no analgesic activity in comparison to the base line of the same group 1 and 2 h post administration.

Thus, it can be concluded that, compounds **IIa**, **IIe**, **IIIa**, **V**, **VIc**, **VIId**, **VIIIc**, **XIc**, **XIIb**, **XIIc**, **XIV**, **XVa**, **XVIb**, **XVIIb** and **XIXb** have significant analgesic activity and compound **XIc** is the most potent one. Results are illustrated by Fig. 2.

3.1.3. Ulcerogenic effect

The ulcerogenic effect of the most active anti-inflammatory and analgesic derivatives: **IIe**, **VIIIc**, **XIc**, **XIIb**, **XIIc**, **XVIb** was evaluated [49]. According to Table 3, it has been found that compounds **IIe**, **XIIb** , **XIIc**, **XVIb** have very little ulcerogenic effect with better safety margin in comparison to indomethacin. Interestingly, compound **XIc** exhibited no ulcerogenic effect in all of the experimental animals. On the other hand compound **VIIIc** resulted in ulcer lesions in many of the experimental rats.

Therefore, the potential medicinal value of these compounds as anti-inflammatory and analgesic agents, that they have better safety margin than indomethacin on gastric mucosa. Results are illustrated by Fig. 3.



Scheme 2.

3.2. Molecular docking study

3.2.1. Aim of work

The aim of this work was to study the crystal structure of COX-2 and to rationalize the obtained biological data and explain the possible interactions that might take place between the tested derivatives and COX-2 enzyme in comparing to Indomethacin® in order to obtain the anti-inflammatory effect. First of all the main interactions of Indomethacin® with COX-2 were determined and we found that the main residue is Tyr 355 (–OH group) forming interactions with the carboxylate group of Indomethacin in a distance equal to 1.03 Ao. From the following Fig. 4, we can also determine the other residues present in the pocket and can be involved in the interactions such as Arg 120, Leu 531, Val 349, and Ser 353 (Fig. 5).

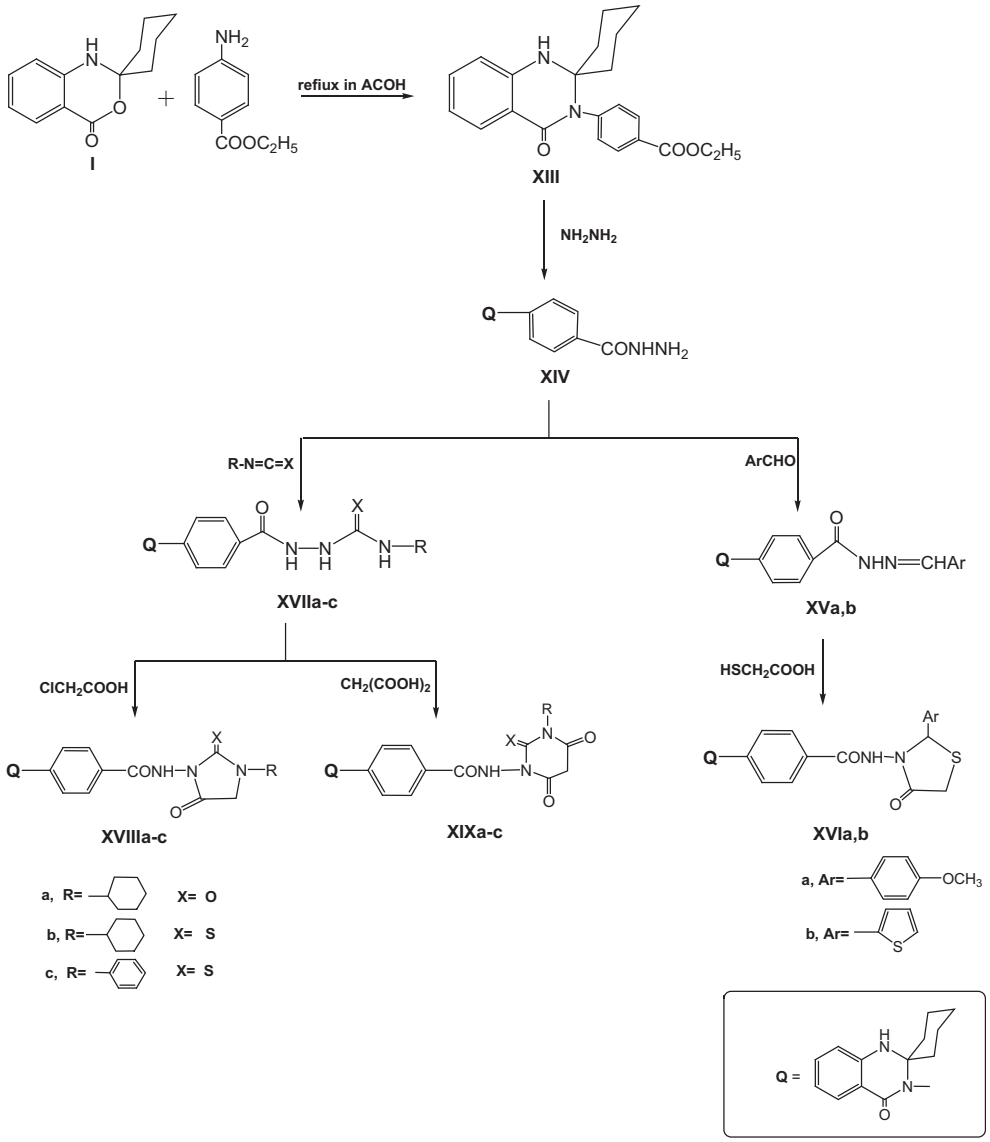
3.2.2. AutoDock binding affinities of the synthesized compounds into COX-2

The crystal structure was downloaded (pdb code: 4COX) and our compounds were docked into the active site using plants software [50]. Compounds (**IIe**, **VIId**, **VIIIc**, **XIb**, **XIc**, **XIIb**, **XIIc** ,**XIII**, **XIV**, **XVIb**, **XVIIIb**) were ranked after docking according to their docking scores and were visualised inside the pocket to view their fitting and closure to the main residues. As we can see in the following Fig. 6, the most active compound **XVIb** which has the best anti-inflammatory action was found to be the best in fitting, and has the best docking score as well and we can see the possible interactions that formed with Arg 120 and Tyr 355 and Ser 530 (Figs. 7–9).

3.2.3. Measurement of the affinities

The affinity of any small molecule can be considered as a unique tool in the field of drug design. As there is a relationship between the affinity of organic molecules and the free binding energy and this can contribute in prediction and interpretation of the activity of organic compounds toward a specific target protein. Here we used Autodock Vina program [51,52] for further docking and to obtain the affinities and RMSD of compounds that have shown anti-inflammatory effect and to compare these data with that was obtained from Indomethacin docking. The following Table 4 illustrates these results.

All compounds were saved as pdbqt format and docking with Autodock Vina was performed according to the specified condition in which the grid box was adjusted to have center_x = 24.15, center_y = 22.8, and center_z = 12.9 by these centres the pocket with the main residues were involved inside the box.



Scheme 3.

3.3. Results and discussion

Autodock vina allows the flexible docking of ligands into its site of action. It has the ability to use all the rotatable bonds of the ligands to give a number of conformations from which the best mode could be achieved. In the analysis of docking results we tried to find a correlation between the biological results and docking studies. Compound **XVIb** which has the highest anti-inflammatory effect has the nearest RMSD value to that of Indomethacin. While all compounds have nearly the same affinity toward the target, we can use RSMD as a tool to compare between them, and this can give us a reasonable description for its good anti-inflammatory activity. It was found that compound **XVIb** has two best conformations in which it can interact with Arg 120 –NH_2 group and Tyr 355 –OH group (Fig. 6A) and with Ser 355 –OH group (Fig. 6B). The distance between –CO (benzamide moiety) in **XVIb** and –NH2 group of Arg 120 was found to be 2.3 Ao while the distance between the –CO (4-oxo-1,3-thiazolidine) in **XVIb** and –OH group of Tyr 355 was found to be 1.9 Ao while that between –CO of quinazoline ring and –OH group of Ser 530 was found to be 1.92 Ao which means good distances for interactions and H-bond formation. Ser 530 interacts with its –OH group first, because its –COOH is out of the specified grid box and its –NH2 group just at the margins of the grid box so, the chance for hydroxyl group was higher for interaction. Second, the –NH2 group of Ser 530 is of a different plane from that of –OH which is directed toward the site of action that was selected for docking. Thus compound **XVIb** has the best docking score with plants and good results with Autodock vina comparing to Indomethacin docking results with both programs.

Compound **IIe** was able to interact by its sulfonyl group forming hydrogen bond with –OH group of Tyr 385 (3.49 Ao)(Fig. 10). Other compounds containing –C═O group in their side chains such as **XIV**(Fig. 11) and **XIII** (ester –C═O) had possible hydrogen bond formation with –OH of Tyr 385 with measured distances 2.52 Ao, and 2.76 Ao respectively.

The cyano group in **XIb** was observed to have an interaction with Tyr 385 with 2.46 Ao (Fig. 12). While other compounds such as **VIId**, **VIIIc**, **XIc**, **XIIb** and **XIIc** did not show any observed bonding or interactions. Finally, the –C═O group of thioxo-imidazolidinone enabled compound **XVIIIb** to form hydrogen bond with –OH of Tyr 355 at distance equal 1.91 Ao.

Table 1
Antiinflammatory effect.

Groups	Oedeama volume (ml)			
	1 h	2 h	3 h	4 h
Control	57.3 ± 6.8	94.4 ± 8.5	100.3 ± 3.3	92.3 ± 3.3
IIa	57.7 ± 5.0	78.9 ± 3.6	87.7 ± 0.8	85.7 ± 4.4
IIe	60.4 ± 7.1	74.1 ± 5.4[a]	80.1 ± 6.0[a]	77.8 ± 5.5[a]
IIIa	67.5 ± 7.7	83.4 ± 5.2	88.6 ± 5.3	94.6 ± 7.4
V	54.6 ± 5.8	80.9 ± 7.0	81.5 ± 2.3	79.9 ± 8.3
VIc	60.4 ± 6.8	93.8 ± 5.2	94.8 ± 0.2	78.2 ± 5.5
VIId	49.9 ± 2.5	88.7 ± 6.1	83.5 ± 3.7	73.4 ± 2.1[a]
VIIIc	57.9 ± 7.2	65.4 ± 8.8[a]	66.4 ± 6.9[a]	68.6 ± 6.3[a]
VIIId	52.2 ± 3.8	79.1 ± 6.9	82.5 ± 8.0	91.6 ± 5.8
XIb	85.4 ± 3.1	74.6 ± 7.5[a]	88.0 ± 7.2	89.9 ± 2.1
XIc	49.4 ± 7.1	71.8 ± 6.7[a]	76.4 ± 4.8[a]	82.2 ± 5.2
XIIb	66.4 ± 7.5	78.2 ± 3.5[a]	81.3 ± 3.3	87.1 ± 2.1
XIIc	61.0 ± 6.6	66.6 ± 5.9[a]	68.6 ± 7.0[a]	72.4 ± 7.4[*]
XIII	44.2 ± 5.1	59.6 ± 4.7[a]	67.8 ± 3.3[a]	81.9 ± 3.2
XIV	57.5 ± 6.3	85.7 ± 2.8	82.1 ± 1.3	75.6 ± 5.1[a]
XVa	72.4 ± 4.9	88.0 ± 9.5	88.7 ± 6.5	90.7 ± 5.4
XVIb	56.2 ± 9.9	65.1 ± 7.5[a]	55.9 ± 10.6[a]	54.7 ± 7.2[a]
XVIIb	65.1 ± 7.2	82.1 ± 6.9	83.9 ± 2.3	79.4 ± 7.3
XVIIIb	54.9 ± 6.2	66.7 ± 6.9[a]	77.2 ± 6.6[a]	79.6 ± 4.9
XIXb	61.7 ± 5.0	79.3 ± 3.0	85.1 ± 3.6	78.6 ± 8.3
Indomethacin	49.8 ± 5.3	42.9 ± 5.1[a]	45.9 ± 4.6[a]	46.9 ± 5.8[a]

[a] $P < 0.05$: Statistically significant from the control using one way ANOVA (Two sided Dunnett as Post Hoc test).

Table 2
Analgesic effect.

Group	Reaction time (sec.)		
	Basal	1 h	2 h
Control	12.2 ± 0.63	12.1 ± 0.83	12.2 ± 1.18
IIa	9.5 ± 0.68	17.5 ± 1.33[a]	17.2 ± 1.11[a]
IIe	10.9 ± 1.03	19.8 ± 1.59[a]	21.6 ± 1.54[a]
IIIa	11.0 ± 0.91	19.1 ± 1.46[a]	19.2 ± 1.00[a]
V	9.1 ± 1.00	17.0 ± 1.11[a]	17.3 ± 1.13[a]
VIc	9.6 ± 1.11	17.1 ± 1.52[a]	17.0 ± 1.25[a]
VIId	9.5 ± 0.49	17.0 ± 1.89[a]	9.32 ± 0.89
VIIIc	12.9 ± 0.95	15.0 ± 1.02	20.5 ± 1.19[a]
VIIId	9.2 ± 0.83	10.9 ± 0.56	15.3 ± 1.17
XIb	11.8 ± 0.82	13.5 ± 0.75	11.5 ± 0.85
XIc	15.1 ± 1.34	24.9 ± 1.38[a]	19.4 ± 0.61[a]
XIIb	13.0 ± 0.85	24.1 ± 1.65[a]	17.8 ± 2.64[a]
XIIc	10.3 ± 1.03	14.6 ± 0.93	18.4 ± 1.47[a]
XIII	12.0 ± 0.92	14.6 ± 1.24	15.6 ± 1.02
XIV	9.4 ± 0.51	17.0 ± 0.43[a]	13.6 ± 1.14
XVa	13.3 ± 1.33	16.9 ± 1.18	18.1 ± 1.36[a]
XVIb	12.6 ± 1.13	21.0 ± 2.47[a]	18.4 ± 0.69[a]
XVIIb	9.3 ± 0.94	17.2 ± 1.66[a]	18.0 ± 1.23[a]
XVIIIb	8.9 ± 0.73	13.0 ± 1.03	16.1 ± 1.20
XIXb	9.4 ± 0.94	17.1 ± 1.66[a]	17.6 ± 1.45[a]
Tramadol	13.1 ± 0.78	17.6 ± 0.32[a]	18.2 ± 0.28[a]

Values represent the mean ± S.E. of six animals for each groups.
[a] $P < 0.05$: Statistically significant from Control. (Dunnett's test).

4. Conclusion

This study includes the synthesis of three series of novel derivatives of spiro quinazoline-2,1′-cyclohexan-4(1H)-ones attached to various aromatic and/or heterocyclic ring systems such as: benzenesulfonamide, pyrazoline, oxazoline, pyrimidin-2-thione, 2-oxo(imino)pyridine, imidazoline and thiazolidinone.

Different nineteen derivatives were evaluated as anti-inflammatory and analgesic agents in experimental animals. It has been found that the derivatives **IIe**, **VIIIc**, **XIc**, **XIIb**, **XIIc**, **XVIb** exhibited the dual pharmacological activities with superior gastrointestinal safety profile when compared to indomethacin except **VIIIc** which resulted in ulcer lesions in many of the experimental rats. Surprisingly, compound **XIc** exhibited no ulcerogenic effect in all of the experimental animals. Thus, it can be concluded that benzenesulfonamide moiety, pyridine and thiazolidinone ring systems are important for both anti-inflammatory and analgesic activity of potent safety margin profiles towards G.I.T.

We have also designed the top eleven anti-inflammatory quinazoline compounds (**IIe**, **VIId**, **VIIIc**, **XIb**, **XIc**, **XIIb**, **XIIc** ,**XIII**, **XIV**, **XVIb**, **XVIIIb**) that were docked into the same pocket of Indomethacin in COX-2 and have shown good docking results and good fitting into the active site. It has been found that these compounds have good antiiflammatory effect comparing to Indomethacin. By the use of molecular modeling we realized the mechanism of their effects that could be their interactions with the same residues that interact with Indomethacin.

5. Experimental

5.1. Chemistry

All melting points are uncorrected, elemental analyses were carried out at the Micro-analytical Laboratory, Central Services Laboratory, National Research Centre, Dokki, Cairo. Infrared spectra were recorded on FT/IR-330E, Fourier transform, Infrared spectrometer at cm^{-1} scale using KBr discs. ^1H NMR spectra were determined using JEOL EX-270 or JEOL ACA500 NMR spectrometers and measured in δ scale using TMS as an internal standard. Mass spectra were measured using mass spectrometer Finnigan MAT

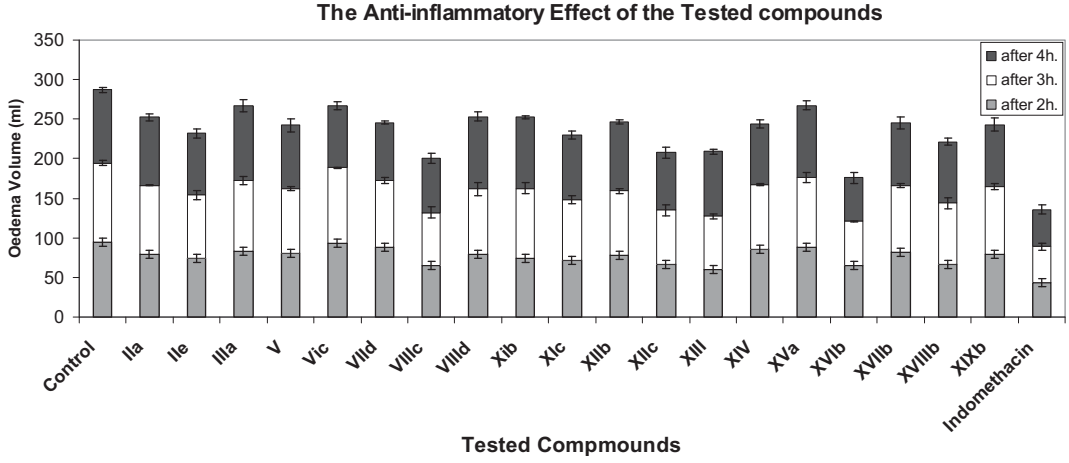


Fig. 1.

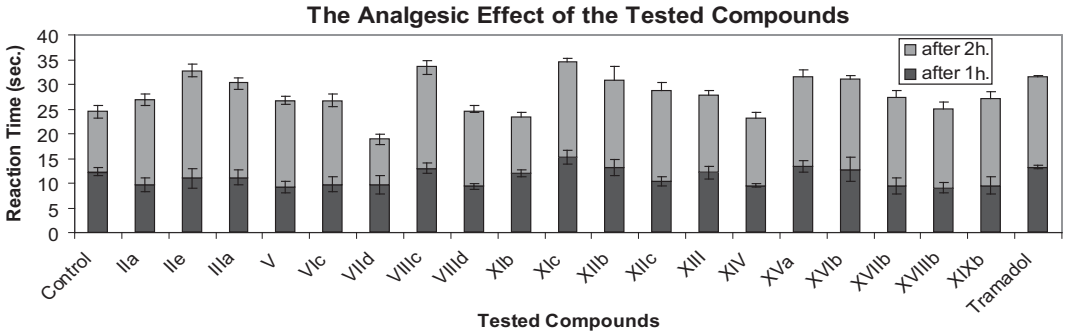


Fig. 2.

SSQ-7000 and GCMS-QP 1000EX Shimadzu Gas Chromatography MS Spectrometer. All reactions were followed up by TLC (aluminum sheets, Merck plates) using $CHCl_3/CH_3OH$ (9:1 v/v) eluent and detected by UV lamp.

*5.1.1. Spiro [2H-3,1-benzoxazine-2,1′-cyclohexan]-4(1H)-one (**I**)*

This compound was prepared according to a reported method [39,40]. mp 143 °C.

*5.1.2. Spiro [(2H, 3H)-3(4-substituted aminosulfonylphenyl)-quinazoline-2,1′-cyclohexan]-4(1H) ones (**IIa–e**)*

General method: a mixture solution of **I** (2.17 g, 10 mmol) and (10 mmol) of the appropriate sulfa drug namely, sulfanilamide, sulfaguanidine, sulfamethoxazole, sulfapyridine and sulfadiazine in 20 mL glacial acetic acid containing (1.64 g, 20 mmol) sodium acetate anhydrous, was refluxed for 15 h. Upon pouring on crushed ice/water, white crystals were obtained, filtered, washed with water and recrystallized from proper solvent.

*5.1.2.1. Spiro[(2H,3H)-3(4-aminosulfonylphenyl)quinazoline-2,1′-cyclohexan]4(1H)one (**IIa**).* Crystallized from isopropanol to give white crystals, mp 203–205 °C, yield 60%. Analysis for $C_{19}H_{21}N_3O_3S$, M.wt.(371.47). Calced.: %C, 61.43; H, 5.69; N, 11.31; S, 8.63. Found: %C, 61.28; H, 5.29; N, 11.93; S, 8.38. IR (KBr, cm^{-1}): 3477, 3470 (NH, NH_2), 3066 (CH-aromatic), 2890 (CH-aliphatic), 1680 (CO,quinazolinone) and 1340 (SO_2NH). MS (*m*/*z*): M^+ 371 (30%) and 119 (100%).

*5.1.2.2. Spiro[(2H,3H)-3(4-guanidosulfonylphenyl)quinazoline-2,1′-cyclohexan]-4(1H)one (**IIb**).* Crystallized from ethanol to give yellowish crystals, mp 256–258 °C, yield; (65%). Analysis for $C_{20}H_{23}N_5O_3S$, M.wt.(413.50). Calced.: %C, 58.09; H, 5.60; N, 16.93; S, 7.75. Found: %C, 57.84; H, 5.81; N, 16.61; S, 7.82. IR (KBr, cm^{-1}): 3467, 3432, 3338, 3211(3NH,NH_2), 1681 (CO, quinazolinone) and 1315 (SO_2NH). ^1H NMR (DMSO-d_6 ,δ ppm): 2.02 (s, 10H, cyclohexane ring), 4.31, 6.81, 9.50 10.20 (4s, 2H, 3H, NH_2, 3NH, exchangeable with D_2O) and 7.10–8.00 (m, 8H, aromatic-H). MS (*m*/*z*): M^+ 413 (5%) and214 (100%).

*5.1.2.3. Spiro [(2H,3H)-3(4-(2′-methyloxazole) sulfonylphenyl) quinazoline-2,1′-cyclohexan]-4(1H) one (**IIc**).* Crystallized from isopropanol/pet.ether to give yellowish crystals, mp 230–232 °C, yield (65%). Analysis for $C_{23}H_{24}N_4O_4S$, M.wt.(452.54), Calced.: %C, 61.04; H, 5.34; N, 12.38; S, 7.08. Found: %C, 61.29; H, 5.22; N, 12.63; S, 7.26. IR (KBr, cm^{-1}): 3471, 3343 (2NH), 3031(CH-aromatic), 1680 (CO, quinazolinone) and 1316 (SO_2NH). ^1H NMR (DMSO-d_6 ,δ ppm): 2.02 (s, 10H, cyclohexane ring), 2.30 (s, 3H, CH_3), 6.82, 10.20 (2s, 2H, 2NH, exchangeable with D_2O) and 7.10–8.00(m, 8H, aromatic-H and 1H, isoxazole ring). MS (*m*/*z*): M^+ 452 (4%) and 62 (100%).

*5.1.2.4. Spiro [(2H,3H)-3(4-(4′pyridinyl)-aminosulfonylphenyl) quinazoline-2,1′-cyclohexan]-4(1H) one (**IId**).* Crystallized from isopropanol/pet.ether to give white crystals, mp 212–214 °C, yield (60%). Analysis for $C_{24}H_{24}N_4O_3S$, M.wt.(448.55). Calced.: %C, 64.26; H, 5.39; N, 12.49; S, 7.14. Found: %C, 64.37; H, 5.51; N, 12.62; S, 7.46; IR (KBr, cm^{-1}): 3355, 3349 (2NH), 3034 (CH-aromatic), 2936 (CH-alicyclic), 1695 (CO, quinazolinone) and 1327 (SO_2NH). MS (*m*/*z*): M^+ 448 (10%) and 93 (100%).

*5.1.2.5. Spiro [(2H,3H)-3(4-(2′-diazine)aminosulfonylphenyl) quinazoline-2,1′-cyclohexan]-4(1H) one (**IIe**).* Crystallized from isopropanol to give brown crystals, mp 203–205 °C, yield (60%). Analysis for $C_{23}H_{23}N_5O_3S$, M.wt.(449.53), Calced.: %,C, 61.45; H, 5.15; N, 15.57; S, 7.13. Found: %C, 61.63; H, 5.48; N, 15.72; S, 7.46. IR (KBr, cm^{-1}): 3414, 3320 (2NH), 3028 (CH-aromatic), 1680 (CO, quinazolinone), 1620 (C=N) and 1325 (SO_2NH). MS (*m*/*z*): M^+ 449 (24%) and 255(100%).

*5.1.3. Spiro[(2H,3H)-3(4-substituted sulfonylurea/(thiourea)phenyl) quinazoline-2,1′-cyclohexan]-4(1H) ones (**IIIa–c**)*

General method: a mixture of IIa (1.85 g, 5 mmol) and anhydrous potassium carbonate (1.38 g, 10 mmol,) in dry acetone (50 mL) was refluxed with continuous stirring for 1.5 h. While hot, a solution of the appropriate iso/isothiocynate derivatives namely: ethyl-isothiocyanate, cyclohexylisocyanate and phenyl isocyanate (7.5 mmol) in dry acetone was added dropwisely and the reflux was continued for further 18 h. The excess acetone was removed under reduced pressure and the obtained solid residue was washed with water, filtered and recrystallized from the proper solvent.

*5.1.3.1. Spiro [(2H,3H)-3(4-ethyl-sulfonylthioureaphenyl)quinazoline-2,1′-cyclohexan]-4(1H) one (**IIIa**).* Crystallized from ethanol to give yellowish brown crystals, mp170–172 °C, yield (70%). Analysis for $C_{22}H_{26}N_4O_3S_2$, M.wt.(458.61). Calced.: %C, 57.61; H, 5.71; N, 12.21; S,13.98. Found: %C, 57.82; H, 5.82; N, 12.52; S,14.05. IR (KBr,

Table 3
Ulcerogenic effect.

Group	Ulcer index		No. Of rats with ulcer/5
	No. of ulcer	Severity of ulcer	
Control (ethanol)	8.2 ± 0.86	19.4 ± 2.20	5
IIe	0.4 ± 0.40[a]	0.6 ± 0.60[a]	1
VIIIc	7.0 ± 1.64	10.4 ± 2.86	5
XIc	0.0 ± 0.00[a]	0.0 ± 0.00[a]	0
XIIb	2.6 ± 1.66[a]	3.2 ± 1.96[a]	2
XIIc	1.6 ± 1.36[a]	2.0 ± 1.76[a]	2
XVIb	0.4 ± 0.40[a]	0.4 ± 0.40[a]	1
Indomethacin	5.8 ± 1.77	9.8 ± 3.23[a]	5

Values represent the mean ± S.E. of five animals for each group.
[a] $P < 0.05$: Statistically significant from ethanol treated rats. (Kruskal Wallis, followed by Mann Whitney test).

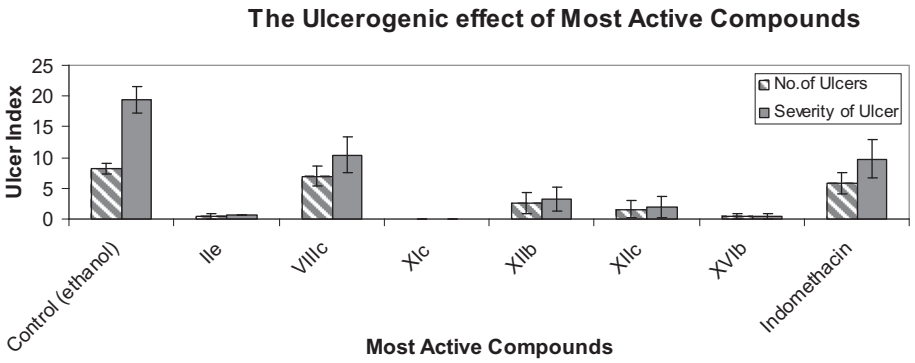


Fig. 3.

cm^{-1}): 3340, 3333, 3247 (3NH), 1710 (CO), 1320 (SO_2NH) and 1160 (C═S). ^1H NMR ($CDCl_3$, δ ppm): 1.20 (t, 3H, CH_3, ethyl group), 1.81 (s, 10H, cyclohexane ring), 3.62 (q, 2H, CH_2, ethyl group), 7.20–8.00 (m, 8H, aromatic-H) and 6.70, 9.25 and 10.20 (3s, 3H, 3NH, exchangeable with D_2O). MS (*m*/*z*): M^+ 458 (5%) and 172 (100%).

*5.1.3.2. Spiro [(2H,3H)-3(4-cyclohexylsulfonylureaphenyl) quinazoline-2,1′-cyclohexan]-4(1H) one. (**IIIb**).* Crystallized from ethanol to give white crystals, mp 226–228 °C, yield (75%). Analysis for $C_{26}H_{32}N_4O_4S$, M.wt.(496.64). Calced.: %C, 62.88; H, 6.49; N, 11.28; S, 6.45. Found: %C, 63.04; H, 6.40; N, 11.53; S, 6.37. IR (KBr, cm^{-1}): 3320, 3310, 3250 (3NH), 2930 (CH-alicyclic), 1710, 1650 (2CO) and 1311 (SO_2NH). ^1H NMR ($CDCl_3$, δ ppm): 1.20–1.11 (m, 6H, cyclohexyl), 1.50–1.50 (m, 4H, cyclohexyl), 2.00(s, 10H, cyclohexane ring), 4.20 (m, 1H, CH–N), 7.02–7.92 (m, 8H, aromatic-H), 6.80, 9.10, and10.10 (3s, 3H, 3NH, exchangeable with D_2O). MS (m/z): M^+ 496 (2%), [M + − 2] 494 (20%) and 56 (100%).

*5.1.3.3. Spiro [(2H,3H)-3(4-phenylsulfonylureaphenyl)quinazoline-2,1′-cyclohexan]-4(1H) one (**IIIc**).* Crystallized from ethanol to give yellowish white crystals, mp 163–165 °C, yield (78%). Analysis for $C_{26}H_{26}N_4O_4S$, M.wt.(490.60). Calced.: %C, 63.65; H, 5.34; N, 11.42; S, 6.53. Found: %C, 63.83; H, 5.28; N, 11.63; S, 6.71. IR (KBr, cm^{-1}): 3340, 3299, 3109 (3NH), 2907 (CH-alicyclic), 1689, 1655 (2CO) and 1312 (SO_2NH). MS (*m*/*z*):M^+ 490 (4%) and 172 (100%).

*5.1.4. Spiro [(2H,3H)-3(4-aminosulfonylphenyl)-1-ethyl-quinazoline-2,1′-cyclohexan]-4(1H) one (**IV**)*

A mixture of compound IIa (1.85 g, 5 mmol,) and iodoethane (0.78 g, 5 mmol) in 100 mL ethanol containing KOH (0.28 g, 5 mmol) was refluxed for 6 h. The reaction mixture was concentrated and acidified with diluted HCl. The separated solid was filtered off and recrystallized from ethanol to give white crystals, mp120–122 °C, yield (60%). Analysis for $C_{21}H_{25}N_3O_3S$, M.wt.(399.52). Calced.: %C, 63.13; H, 6.30; N, 10.51; S, 8.02. Found: C, 63.41; H, 6.51; N, 10.31; S, 8.15. ^1H NMR (DMSO-d_6,δ ppm): 0.90–1.00 (t, 3H, CH_3, ethyl group), 2.11 (s, 10H, cyclohexane ring), 3.00–3.21 (q, 2H, CH_2, ethyl group), 7.00–8.00 (m, 8H, aromatic-H) and 4.55 (s, 2H, NH_2 exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 15.2 (CH_3), 39.64 (CH_2), 39.8–40.00 (spiro cyclohexyl carbons), 71.8 (spiro head carbon), 119.17–144.23 (aromatic carbons) and 155 (CO, quinazolinone ring). MS (*m*/*z*): M^+ 399 (5%) and 254 (100%).

*5.1.5. Spiro [(2H, 3H)-3-(4-acetylphenyl) quinazoline-2,1′-cyclohexan]−4(1H)-one (**V**)*

A mixture of the benzoxazine derivatives **I** (2.17g, 10 mmol,) and *p*-aminoacetophenone (1.35 g, 10 mmol) in glacial acetic acid (20 mL) in the presence of sodium acetate (1.64 g, 20 mmol,) was refluxed for 6 h. The reaction was poured onto ice/water and neutralized with diluted solution of NaOH. The obtained product was filtered off, washed with water and recrystallized from ethanol to give yellowish brown crystals, mp 165–167 °C, yield (65%). Analysis for $C_{21}H_{22}N_2O_2$, M.wt. (334.41). Calced.: %C, 75.42; H, 6.62; N,8.37. Found,: %C, 75.20; H, 6.84; N, 8.02. IR (KBr, cm^{-1}): 3423 (NH), 3053 (CH, aromatic), 2930 (CH, alicyclic) and 1673,1670 (2CO). ^1H NMR (DMSO-d_6,δ ppm): 2.12 (s,10H, cyclohexane ring), 2.60 (s, 3H, $COCH_3$), 7.20–8.00 (m, 8H, aromatic-H) and 10.20 (s, 1H, NH,exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 26.32 (CH_3), 38.78–40.32 (spiro cyclohexane carbons), 71.8 (spiro head carbon), 118.04–143.61 (aromatic carbons), 168 (CO,quinazoline ring), 196.39 (CO, acetyl). MS (*m*/*z*): M^+ 334 (14%), and 119 (100%).

*5.1.6. Spiro [(2H, 3H)-3-(substituted acryloylphenyl)quinazoline-2,1′-cyclohexan]-4(1H)-ones (**VIa–c**)*

General method: a mixture of compound **V** (3.34 g, 10 mmol) and the appropriate aldehyde namely: benzaldehyde, *N*,*N*-dimethyl aminobenzaldehyde and furan-2-carboxaldehyde (10 mmol) in 5% ethanolic sodium hydroxide solution (40 mL) was refluxed while stirring for 15 h. The reaction mixture was poured onto ice/cold water and neutralized with dil. HCl. The formed precipitate was filtered off and recrystallized from the proper solvent to give compounds **VIa**,**b**, respectively.

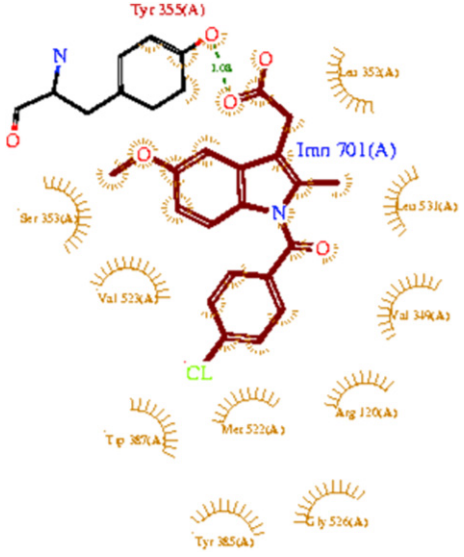


Fig. 4. Showing Indomethacin interaction with Tyr 355 –OH group with the –CO group of Indomethacin carboxylic group with distance 1.03 Ao. Image was taken using Ligplot.

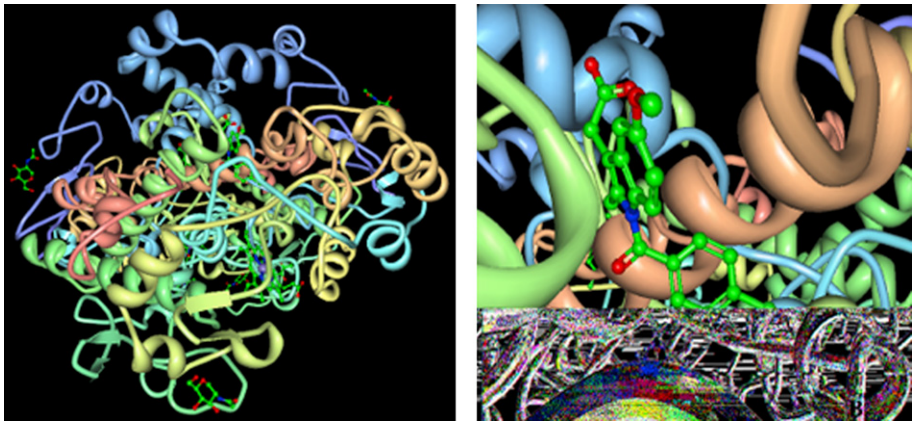


Fig. 5. (A) Shows the 3D crystal structure of COX-2 complexed with Indomethacin (pdb: 4COX). (B) Indomethacin (In green color) fitting into the pocket. The structure was viewed by PDB simpleViewer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

*5.1.6.1. Spiro [(2H,3H)-3-(phenylacryloylphenyl) quinazoline2,1′-cyclohexan]-4(1H)-one(**VIa**).* Crystallized from ethanol to give brown crystals, mp 135–137 °C, yield(60%).. Analysis for $C_{28}H_{26}N_2O_2$, M.wt.(422.52). Calced.: %C, 79.59; H, 6.20; N, 6.63. Found: %C, 79.38; H, 6.48; N, 6.41. IR (KBr, cm^{-1}): 3330 (NH), 3044(CH, aromatic), 1670, 1650 (2CO). ^{1}H NMR (DMSO-d_6,δ ppm): 2.04 (s, 10H, cyclohexane ring), 6.68–6.75 (d,d, 2H, CH═CH, J = 5.6 Hz), 7.55–8.10 (m,13H, aromatic-H) and 10.12 (s, 1H, NH, exchangeable with D_2O). MS (m/z): M^+ 422 (5%) and 120 (100%).

*5.1.6.2. Spiro[(2H,3H)-3-(N,N-dimethylaminophenyl–acryloylphenyl)-quinazoline2,1′-cyclo hexan]-4(1H)-one (**VIb**).* Crystallized from ethanol to give yellow crystals, mp 145 °C, yield (70%). Analysis for $C_{30}H_{31}N_3O_2$, M.wt.(465.59).Calced.: %C, 77.39; H, 6.70; N, 9.02. Found: %C, 77.34; H, 6.58; N, 9.33. IR (KBr, cm^{-1}): 3325 (NH) and 1685, 1646 (2CO). ^{1}H NMR (DMSO-d_6,δ ppm): 2.04 (s, 10H, cyclohexane ring), 3.00(s, 6H, $N(CH_3)_2$), 6.68–6.75 (d,d, 2H, CH═CH, J = 5.4 Hz), 7.55–8.10 (m,12H, aromatic-H) and 10.12 (s, 1H, NH, exchangeable with D_2O).MS (m/z): M^+ 465 (5%) and 266 (100%).

*5.1.6.3. Spiro [(2H,3H)-3-(2′furanoylacryloylphenyl)quinazoline2,1′-cyclohexan]-4(1H)-one (**VIc**).* Crystallized from ethanol to give yellowish brown crystals, mp 125–127 °C, yield (85%). Analysis for $C_{26}H_{24}N_2O_3$, M.wt.(412.49). Calced.: %C, 75.70; H, 5.86; N, 6.79. Found: %C, 75.86; H, 5.94; N, 6.55. IR (KBr, cm^{-1}): 3326 (NH), 3044(CH, aromatic), 2995(CH-alicyclic) and 1699, 1641 (2CO). MS (m/z): M^+ 412 (5%) and 120 (100%).

*5.1.7. Spiro [(2H, 3H)-3-(4-(5-aryl)-4,5-dihydro-1H-pyrazol-3-yl-phenyl) quinazoline2,1′ cyclohexan]-4(1H)-ones (**VIIa–d**)*

General method: a mixture of the chalcone **VIa–b** (30 mmol) and hydrazine hydrate 98% (3 mL, 90 mmol) in absolute ethanol was

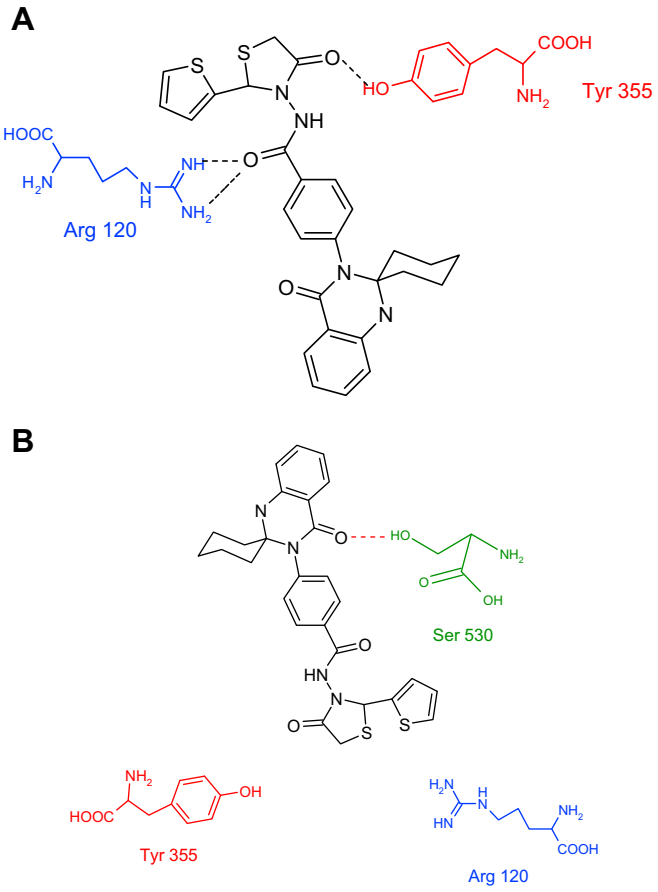


Fig. 6. A) Possible interactions of compound **XVIb** with Arg 120 and Tyr 355. B) Possible hydrogen bond formation with –OH of Ser 530.

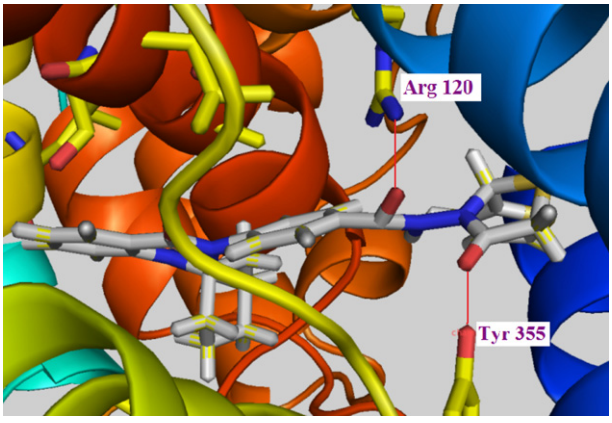


Fig. 7. illustrates compound XVIb (in Stick) and how it fits into the pocket. The protein is presented as cartoon and colored as spectrum.

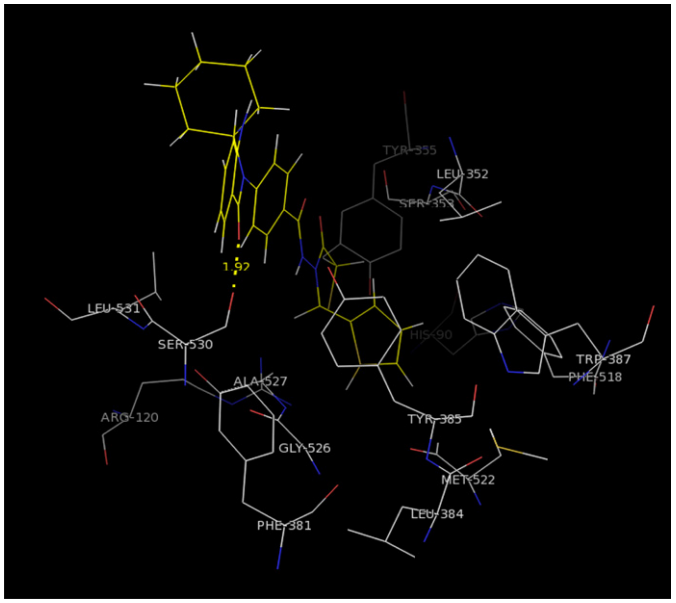


Fig. 8. Distance between –C═O of quinazoline ring and –OH group of **XVIb**.

refluxed for 3 h. Upon cooling, white crystals were obtained, filtered off and recrystallized from the proper solvent to obtain the compounds **VIIa**,**b**, respectively. When the reaction was carried out in glacial acetic acid (20 mL) and refluxed for 4 h, white crystals were obtained upon pouring onto ice/water. The products were filtered off and recrystallized from the proper solvents to obtain the desired derivatives **VIIc**,**d**, respectively.

*5.1.7.1. Spiro [(2H, 3H)-3-(4-(5-p-N,N-dimethylaminophenyl)-4,5-dihydro-1H-pyrazol-3-yl-phenyl)quinazoline 2, 1′-cyclohexan]-4(1H)-one (**VIIa**).* Crystallized from methanol to give orange crystals, mp 115–117 °C, yield (85%). Analysis for $C_{30}H_{33}N_5O$, M.wt.(479.62). Calced.: %C, 75.12; H, 6.93; N, 14.60. Found: %C, 75.48; H, 6.85; N, 14.76. IR (KBr, cm^{-1}): 3337, 3211 (2NH), 1690 (CO) and 1595(C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.00 (s, 10H, cyclohexane ring), 3.00 (s, 6H, $N(CH_3)_2$), 3.40, 3.80 (d,d, 2H, CH_2, pyrazoline ring, $J = 5.6$ Hz), 6.51 (t, 1H, CH, pyrazoline ring), 7.50–8.28 (m, 12H, aromatic-H) and 10.21,11.45 (2s, 2NH, exchangeable with D_2O). MS (m/z): M^+ 479(4%), and 148 (100%).

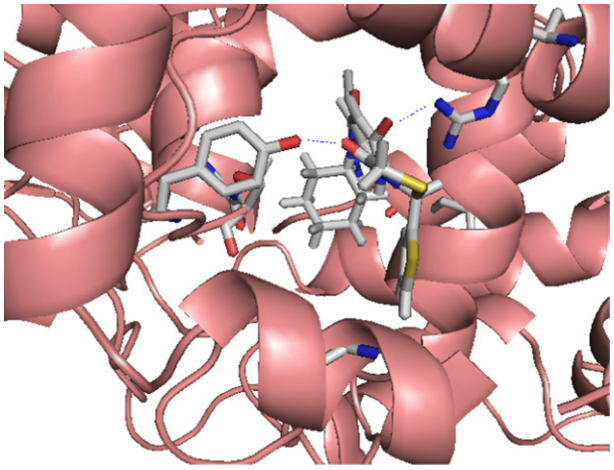


Fig. 9. The possible H-bond formation between both Arg 120 NH2 group and Tyr 355 –OH group and two carbonyl groups in **XVIb**. The protein is shown in catroon view and colored as salmon while both the compound, Tyr, and Arg are shown as sticks.

Table 4
illustrates the calculated affinities and RMSD for the top 11 anti-inflammatory compounds and Indomethacin.

Compound	Ranking for biological anti-inflammatory effect	Affinity Kcal/mol	RMSD Deviation from best mode
XVIb	1	−5.4	4.07
XIIc	2	−5.8	9.5
VIIIc	3	−5.5	11.6
IIe	4	−5.2	15.02
XIII	5	−5.6	10.3
XIc	6	−5.7	9.2
XVIIIb	7	−5.4	8.3
XIb	8	−5.9	9.0
XIIb	9	−5.8	9.3
VIId	10	−5.2	9.5
XIV	11	−5.1	7.2
Indomethacin	–	−5.0	4.6

*5.1.7.2. Spiro [(2H, 3H)-3-(4-(5(2′furanoyl))-4,5-dihydro-1H-pyrazol-3-yl-phenyl)quinazoline 2, 1′-cyclohexan]-4(1H)-one (**VIIb**).* - Crystallized from methanol to give yellowish brown crystals, mp 150–152 °C, yield (60%). Analysis for $C_{26}H_{26}N_4O_2$, M.wt.(426.53). Calced.: %C, 73.21; H, 6.14; N, 13.13; Found: %C, 72.99; H, 6.05; N, 13.42. IR (KBr, cm^{-1}): 3325 (2NH), 2995 (CH, alicyclic), 1700 (CO), and 1600(C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.00 (s, 10H, cyclohexane ring), 3.40, 3.80 (d,d, 2H, CH_2, pyrazoline ring, $J = 5.6$ Hz), 6.50 (t, 1H, CH, pyrazoline ring), 7.50–8.28 (m, 11H, aromatic-H) and 10.21,11.45 (2s, 2NH, exchangeable with D_2O). MS (m/z): M^+ 426 (5%) and 279 (100%).

*5.1.7.3. Spiro [(2H, 3H)-3-(4-(5-p-N,N-dimethyl amino phenyl)-4,5-dihydro-1acetyl-pyrazol-3-yl-phenyl)quinazoline 2, 1′-cyclohexan]-4(1H)-one (**VIIc**).* Crystallized from isopropanol to give yellowish brown crystals, mp 243–245 °C,yield (60%). Analysis for $C_{32}H_{35}N_5O_2$, M.wt.(521.66). Calced.: %C, 73.67; H, 6.76; N, 13.42; Found: %C, 73.48; H, 6.69; N, 13.64; IR (KBr, cm^{-1}): 3352 (NH), 3046 (CH, aromatic), 2929 (CH, alicyclic), 1700, 1677 (2C0) and 1608(C═N). MS (m/z) :M^+ 521 (10%) and 74 (100%).

*5.1.7.4. Spiro [(2H, 3H)-3-(4-(5(2′furanoyl))-4,5-dihydro-1acetyl-pyrazol-3-yl-phenyl) quinazoline 2, 1′-cyclohexan]-4(1H)-one (**VIId**).* Crystallized from isopropanol to give brown crystals, mp 245–247 °C, yield (65%). Analysis for $C_{28}H_{28}N_4O_3$, M.wt.(468.56). Calced.: %C, 71.77; H, 6.02; N, 11.95. Found: %C, 72.03; H, 6.21; N, 11.74. IR (KBr, cm^{-1}): 3309 (NH), 3042 (CH, aromatic), 2894 (CH, alicyclic), 1698, 1652 (2CO) and 1560 (C═N). ^{1}H NMR (DMSO-d_6,δ ppm): 2.00 (s,10H, cyclohexane ring), 2.20 (s, 3H, $COCH_3$), 2.90, 3.22

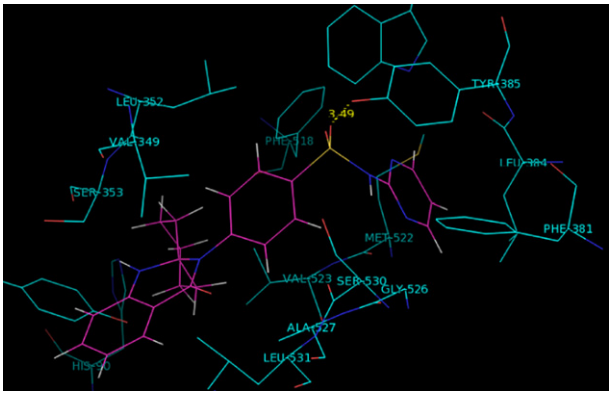


Fig. 10. compound IIe measured distance between sulfonyl group and –OH group of Tyr 385.

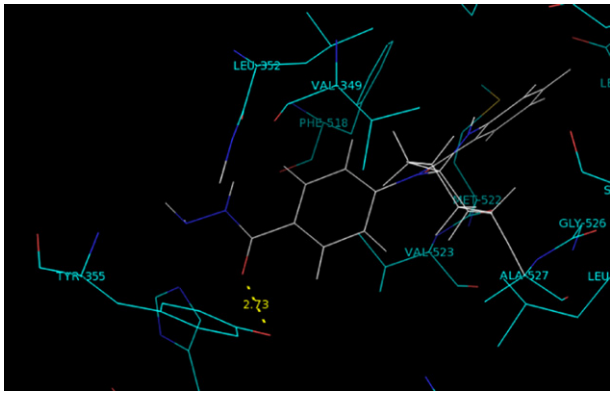


Fig. 11. Hydrogen bond formation between **XIV** –C═O and –OH group of Tyr 355.

(d,d, 2H, CH_2, pyrazoline ring, J = 5.6 Hz), 6.11 (t, 1H, CH of pyrazoline ring), 7.12–8.00 (m, 11H, aromatic-H) and 10.22 (s, 1H, NH). MS (*m*/*z*): M^+ 468 (3%) and 78 (100%).

*5.1.8. Spiro [(2H, 3H)-3-(4-(5-substituted)-4,5-dihydro-1-methyl/phenyl-1H-pyrazol-3-yl-phenyl) quinazoline-2,1′-cyclohexan]-4(1H)-ones (**VIIIa–d**)*

General method: a mixture of the chalcone derivatives **VIa**,**b** (3 mmol) and methyl hydrazine(0.14 g, 3 mmol) or phenyl hydrazine (0.32 g, 3 mmol) in absolute ethanol (30 mL) was refluxed for 6 h. Upon cooling, the obtained product was filtered off and recrystallized from isopropanol to obtain the titled derivative **VIIIa–d**

*5.1.8.1. Spiro [(2H, 3H)-3-(4-(5-N,N-dimethylaminophenyl)-4,5-dihydro-1-methyl-1H-pyrazol-3-yl-phenyl)quinazoline-2,1′-cyclohexan]-4(1H)-one (**VIIIa**).* Crystallized from isopropanol to give yellowish brown crystals, mp 244–246 °C yield (65%). Analysis for $C_{31}H_{35}N_5O$, M.wt. (493.65) Calced.: %C, 75.42; H, 7.14; N, 14.18; Found: %C, 75.33; H, 7.29; N, 14.38. IR (KBr, cm^{-1}): 3385 (NH), 3030 (CH, aromatic), 2930 (CH, alicyclic),1670 (C═O) and 1630(C═N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.00 (s, 10H, cyclohexane ring), 2.31 (s, 3H, CH_3), 3.00 (s, 6H, $N(CH_3)_2$), 3.40, 3.80 (d,d, 2H, CH_2, pyrazoline ring, J = 5.6 Hz), 6.50 (t, 1H, CH, pyrazoline ring), 7.50–8.28 (m, 12H, aromatic-H) and 10.21 (s, NH, exchangeable with D_2O). MS (*m*/*z*):M^+ 493 (10%), and 73 (100%).

*5.1.8.2. Spiro [(2H, 3H)-3-(4-(5-[2′-furanoyl])-4,5-dihydro-1-methyl)-1H-(pyrazol-3-yl-phenyl)quinazoline-2,1′-cyclohexan]-4(1H)-one (**VIIIb**).* Crystallized from isopropanol to give brown crystals, mp 240–242 °C, yield (60%). Analysis for $C_{27}H_{28}N_4O_2$, M.wt. (440.55). Calced.: %C, 73.61; H, 6.40; N, 12.71. Found: %C, 73.72; H, 6.89; N, 12.68. IR (KBr, cm^{-1}): 3367(NH), 2928 (CH, alicyclic), 1677 (CO) and 1604 (C═N). MS (*m*/*z*): [M^+ + 2] 442 (10%) and 73 (100%).

*5.1.8.3. Spiro [(2H, 3H)-3-(4-(5-p-N,N-dimethylaminophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl-phenyl)quinazoline-2,1′-cyclohexan]-4(1H)-one (**VIIIc**).* Crystallized from isopropanol to give brown crystals, mp 160–162 °C, yield (85%). Analysis for $C_{36}H_{37}N_5O$, M.wt.(555.72). Calced.: %C, 77.80; H, 6.71; N, 12.60. Found: %C, 77.73; H, 6.84; N, 12.52. IR (KBr, cm^{-1}): 3312 (NH), 3017 (CH, aromatic), 2930 (CH, alicyclic), 1672 (CO) and 1600 (C═N). MS (*m*/*z*): M^+ 555 (4%) and 53 (100%).

*5.1.8.4. Spiro [(2H, 3H)-3-(4-5-[2′-furanoyl])-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl-phenylquinazoline-2,1′-cyclohexan]-4(1H)-one (**VIIId**).* Crystallized from isopropanol to give dark brown

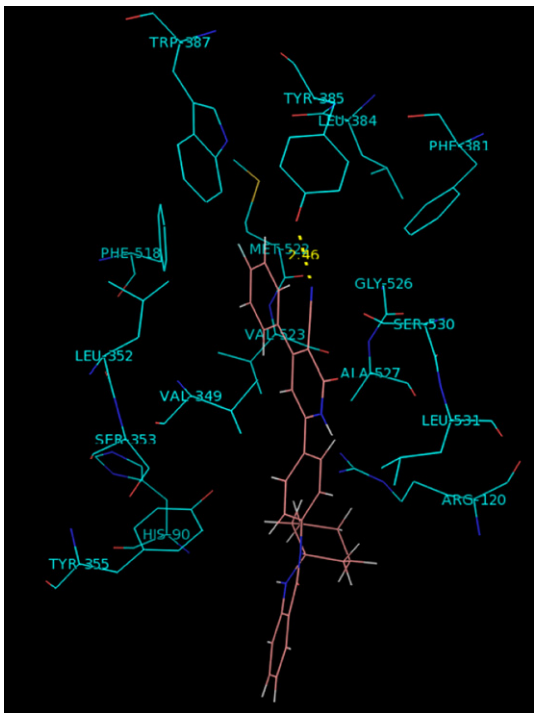


Fig. 12. Possible interactions between cyano group of compound **XIb** and –OH of Tyr 385.

crystals, mp 85–87 °C, yield (60%). Analysis for $C_{32}H_{30}N_4O_2$, M.wt.(502.62). Calced.: %C, 76.46; H, 6.01; N, 11.14. Found: %C, 76.63; H, 6.38; N, 10.92. IR (KBr, cm^{-1}): 3363 (NH), 3037 (CH, aromatic), 2957 (CH, alicyclic), 1677 (CO) and 1628 (C═N). MS (*m*/*z*): M^+ 502 (10%) and 239 (100%).

*5.1.9. Spiro(2H,3H)-3-(4-(5-substituted)-4,5-dihydroisoxazole-3-yl-phenyl)quinazoline2,1′-cyclohexan]-4(1H)-ones (**IXa**,**b**)*

General method: a mixture of compounds **VIa**,**b** (3 mmol) and hydroxylamine hydrochloride (5 mmol) in sodium hydroxide solution (0.5 g NaOH in 0.5 mL water) in ethanol (60 mL) was refluxed for 3 h. The product obtained upon cooling was filtered off, washed with water and recrystallized from the proper solvents to obtain the desired compounds **IXa**,**b** respectively.

*5.1.9.1. Spiro [(2H, 3H)-3-(4-(5-p-N,N-dimethylamino-phenyl)-4,5-dihydroisoxazole-3-yl-phenyl)quinazoline2,1′-cyclohexan]-4(1H)-one (**IXa**).* Crystallized from methanol to obtain yellowish white crystals, mp 250–252 °C, yield (50%). Analysis for $C_{30}H_{32}N_4O_2$, M.wt. (480.61). Calced.: %C, 74.97; H, 6.71; N, 11.65; Found: %C, 74.63; H, 6.59; N, 11.83. IR (KBr, cm^{-1}): 3351(NH), 3042(CH, aromatic), 2920(CH, alicyclic), 1661(CO) and 1620 (C═N). ^{1}H NMR (DMSO-d_6, δ ppm) :1.80 (s, 10H, cyclohexane ring), 2.58–2.62 (d, d, 2H, CH_2, isoxazoline ring, J = 5.4 Hz), 3.00 (s, 6H, $N(CH_3)_2$), 6.44 (t, 1H, CH, isoxazoline ring), 7.00–8.00 (m,11H, aromatic-H) and 10.2 (s, 1H, NH, exchangeable with D_2O).

*5.1.9.2. Spiro [(2H, 3H)-3-(4-(5-[2′-furanoyl])-4,5-dihydroisoxazole-3-yl-phenyl)quinazoline 2,1′-cyclohexan]-4(1H)-one (**IXb**).* Crystallized from methanol to give yellow crystals, mp 110–112 °C, yield (55%). Analysis for $C_{26}H_{25}N_3O_3$, M.wt. (427.51). Calced.: %C, 73.04; H, 5.89; N, 9.82. Found: %C, 73.29; H, 5.71; N, 9.69. IR (KBr, cm^{-1}): 3350 (NH), 3040 (CH, aromatic), 2934 (CH, alicyclic), 1670 (CO), 1610 (C═N). MS (*m*/*z*): M^+ 427 (15%) and 99 (100%).

*5.1.10. Spiro [(2H, 3H)-3-(4-(6-substituted)-1,2-dihydro-2-thiopyrimidin-4-yl-phenyl)-quinazoline2,1′-cyclohexan]-4(1H)-ones(**Xa**,**b**)*

General method: a solution of the chalcone derivatives **VIa**,**b** (3 mmol), thiourea (0.23 g, 3 mmol) and sodium hydroxide (0.1 g) in absolute ethanol (30 mL) was refluxed for 6 h. The reaction mixture was concentrated under vacuum, cooled and neutralized with dilute HCl. The formed product was filtered off and washed with water to yield the **Xa**,**b**, respectively.

*5.1.10.1. Spiro [(2H,3H)–3-(4-(6-N,N–dimethylaminophenyl)-1,2-dihydro-2-thiopyrimidin-4-yl-phenyl)quinazoline2,1′-cyclohexan]-4(1H)-one (**Xa**).* Crystallized from isopropanol to give reddish brown crystals, mp 175–177 °C, yield (71%). Analysis for $C_{31}H_{31}N_5OS$, M.wt.(521.68). Calced.: %C, 71.37; H, 5.98; N, 13.42; S, 6.14. Found: %C, 71.58; H, 6.77; N, 13.23; S, 6.35. IR (KBr, cm^{-1}): 3384, 3351(2NH), 3043 (CH, aromatic), 2924 (CH, alicyclic), 1660 (CO), 1596 (C═N) and 1227 (C═S). MS (*m*/*z*): M^+ 521(10%) and 147(100%).

*5.1.10.2. Spiro [(2H, 3H)-3-(4-(6-[2′-furanoyl])-1,2-dihydro-2-thiopyrimidin-4-yl-phenyl)-quinazoline2,1′-cyclohexan]-4(1H)-one (**Xb**).* Crystallized from methanol to give orange crystals, mp 130–132 °C, yield (50%). Analysis for $C_{27}H_{24}N_4O_2S$, M.wt. (468.59). Calced.: %C, 69.20; H, 5.16; N, 11.95; S, 6.84. Found: %C, 69.53; H, 5.32; N, 12.11; S, 6.77. IR (KBr, cm^{-1}): 3338, 3214 (2NH), 3067 (CH, aromatic), 2918 (CH, alicyclic), 1660 (CO), 1590 (C═N) and 1273 (C═S). MS (*m*/*z*): M^+ 468 (15%) and 112 (100%).

*5.1.11. Spiro (2H, 3H)-3-(4-(4-aryl)-1, 2-dihydro-3-cyano-2-oxo-pyridin-6-yl-phenyl)-quinazoline-2,1′-cyclohexan]-4(1H)-ones (**XIa**–**c**)*

General method: a mixture of the acetyl derivative **V** (3.34 g, 10 mmol), ethyl cyanoacetate (1.13 g, 10 mmol), and the appropriate aldehyde namely: benzaldehyde, *p*-florobenzaldehyde and 2-hydroxybenzaldehyde (10 mmol) and ammonium acetate (6.16 g, 80 mmol) in *n*-butanol (50 mL) was heated under reflux for 6 h. The solid separated upon cooling was filtered off, washed with water and recrystallized from the proper solvent to obtain the desired product **XIa**–**c**, respectively.

*5.1.11.1. Spiro[(2H,3H)–3-(4-(4-phenyl)-1,2–dihydro-3-cyano-2-oxo-pyridin-6-yl-phenyl)-quinazoline-2,1′-cyclohexan]-4(1H)-one (**XIa**).* Crystallized from glacial acetic acid to give bright yellow crystals, mp over 300 °C, yield (57%). Analysis for $C_{31}H_{26}N_4O_2$, M.wt.(486.58). Calced.: %C, 76.52; H, 5.38; N, 11.51. Found: %C, 76.69; H, 5.56; N, 11.49. IR (KBr, cm^{-1}): 3331, 3237 (2NH), 3079 (CH,aromatic), 2965 (CH,alicyclic), 2128 (C≡N), 1660, 1656 (2CO) and 1601(C═N). MS (*m*/*z*): M^+ 486 (16%) and 59 (100%).

*5.1.11.2. Spiro[(2H,3H)-3-(4-(4-p-florophenyl)-1,2-dihydro-3-cyano-2-oxo-pyridin-6-yl-phenyl)quinazoline-2,1′-cyclohexan]-4(1H)-one (**XIb**).* Crystallized from methanol to give yellowish white crystals, mp over 300 °C, yield (50%). Analysis for $C_{31}H_{25}FN_4O_2$, M.wt. (504.57). Calced.: %C, 73.79; H, 4.99; N, 11.10. Found: %C, 73.82; H, 4.72; N, 10.92. IR (KBr, cm^{-1}): 3339, 3263 (2NH), 3065 (CH,aromatic), 2927 (CH,alicyclic), 2213(C≡N), 1665,1656 (2CO) and 1599 (C═N). MS (*m*/*z*) :M^+ 504 (24%) and 307 (100%).

*5.1.11.3. Spiro[(2H,3H)-3-(4-(4-O-hydroxyphenyl)-1,2-dihydro-3-cyano-2-oxo-pyridin-6-yl-phenyl)quinazoline-2,1′-cyclohexan]-4(1H)-one (**XIc**).* Crystallized from methanol to give orange crystals, mp over 300 °C, yield (60%). Analysis for $C_{31}H_{26}N_4O_3$, M.wt.(502.58). Calced.: %C, 74.08; H, 5.21; N, 11.14. Found: %C, 73.99; H, 5.32; N, 11.29. IR (KBr, cm^{-1}): 3331, 3213 (2NH), 2248 (C≡N), 1666, 1650 (2CO) and 1557(C═N). MS (*m*/*z*): [M^++2] 504 (24%), and 62 (100%).

*5.1.12. Spiro (2H, 3H)-3-(4-(4-aryl)-1,2-dihydro-3-cyano-2-imino-pyridin-6-yl-phenyl)- quinazoline-2,1′-cyclohexan]-4(1H)-ones (**XIIa**–**c**)*

General method: a mixture of the acetyl derivative **V** (3.34 g; 10 mmol), malononitrile (0.66 g; 10 mmol) and the appropriate aldehyde namely: benzaldehyde, *p*-florobenzaldehyde and 2-hydroxybenzaldehyde (10 mmol) and ammonium acetate (6.16 g; 80 mmol) in *n*-butanol (50 mL) was heated under reflux for 6 h. The solid formed upon concentrating the solvent was filtered off, washed with water and recrystallized from the proper solvent to obtain the desired product **XIIa**–**c**, respectively.

*5.1.12.1. Spiro[(2H,3H)-3-(4-(4-phenyl)-1,2-dihydro-3-cyano-2-imino-pyridin-6-yl-phenyl) quinazoline-2,1′-cyclohexan]-4(1H)-one (**XIIa**).* Crystallized from glacial acetic acid to give yellow crystals, mp 139–141 °C, yield (50%). Analysis for $C_{31}H_{27}N_5O$, M.wt. (485.58). Calced.: %C, 76.67; H, 5.60; N, 14.42. Found: %C, 76.49; H, 5.48; N, 14.64. IR (KBr, cm^{-1}): 3445, 3337, 3218 (3NH), 3061 (CH, aromatic), 2936 (CH, alicyclic), 2196 (C≡N), 1670 (CO) and 1624 (C═N). MS (*m*/*z*): M^+ 486 (18%) and 190 (100%).

*5.1.12.2. Spiro[(2H,3H)-3-(4-(4-p-florophenyl)-1,2-dihydro-3-cyano-2-imino-pyridin-6-yl-phenyl) quinazoline-2,1′-cyclohexan]-4(1H)-one (**XIIb**).* Crystallized from methanol to give orange crystals, mp 132–134 °C, yield (53%). Analysis for $C_{31}H_{26}FN_5O$, M.wt.(503.58). Calced.: %C, 73.93; H, 5.20; N, 13.90. Found: %C, 74.15; H, 5.43; N, 13.73. IR (KBr, cm^{-1}): 3438, 3346, 3237 (3NH), 3087 (CH, aromatic), 2928 (CH, alicyclic), 2204 (C≡N), 1670 (CO) and 1612 (C═N). MS (*m*/*z*): M^+ 503 (14%) and 62 (100%).

*5.1.12.3. Spiro[(2H,3H)-3-(4-(4-hydroxyphenyl)-1,2-dihydro-3-cyano-2-imino-pyridin-6-yl-phenyl)quinazoline-2,1′-cyclohexan]-4(1H)-one (**XIIc**).* Crystallized from methanol to give light brown crystals, mp 218–220 °C, yield (57%). Analysis for $C_{31}H_{27}N_5O_2$, M.wt. (501.58). Calced.: %C, 74.23; H, 5.42; N, 13.96. Found: %C, 74.49; H, 5.33; N, 13.68. IR (KBr, cm^{-1}): 3438, 3346, 3237 (3NH), 3087 (CH, aromatic), 2928 (CH, alicyclic), 2204 (C≡N), 1670 (CO) and 1612 (C═N). MS (*m*/*z*) :M^+ 502 (8%) and 69 (100%).

*5.1.13. Ethyl-4-[spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-benzoate (**XIII**)*

A mixture of the benzoxazine derivative I (2.17 g; 10 mmol) and ethyl-*p*-aminobenzoate (1.65 g; 10 mmol) in glacial acetic acid (30 mL) containing sodium acetate anhydrous (1.64 g; 20 mmol) was refluxed for 8 h. Upon pouring on ice/water and neutralization with dilute NaOH solution, white precipitate was obtained which was filtered off, washed with H_2O and recrystallized with isopropanol to obtain the desired ester derivative XIII. mp 70–72 °C, yield (75%**).** Analysis for $C_{22}H_{24}N_2O_3$, M.wt. (364.44). Calced.: %C, 72.50; H, 6.63; N, 7.68. Found: %C, 72.40; H, 6.70; N, 7.60. IR (KBr, cm^{-1}): 3291 (NH), 3066(CH, aromatic), 2990 (CH, alicyclic) and 1690, 1685 (2CO). ^1H NMR (DMSO-d_6, δ ppm): 1.21(t, 3H, CH_3, ethyl group), 2.09 (s, 10H, cyclohexane ring), 4.20 (q, 2H, CH_2, ethyl group) and 7.11–8.02 (m, 8H, aromatic-H) and 10.21 (s, 1H, NH, exchangeable with D_2O). MS (*m*/*z*): M^+ 364 (10%) and 120 (100%).

*5.1.14. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-benzoic acid hydrazide (**XIV**)*

A solution of the ester derivative **XIII** (3.64 g; 10 mmol) and hydrazine hydrate 98% (1.6 g; 50 mmol) in absolute ethanol (20 mL) was refluxed for 6 h upon cooling, the formed precipitate was filtered off and recrystallized from ethanol to give the hydrazide

derivative XIV. mp 300–302 °C, yield (67%). Analysis for $C_{20}H_{22}N_4O_2$, M.wt. (350.42). Calced.: %C, 68.55; H, 6.32; N, 15.98. Found: %C, 68.70; H, 6.13; N, 15.90. IR (KBr, cm^{-1}): 3311, 3219 (NH, NH_2), 3046 (CH, aromatic), 2931(CH, alicyclic), 1672 (CO, quinazoline ring) and 1640 (CO, amide). ^1H NMR (DMSO-d_6,δ ppm): 2.00 (s, 10H, cyclohexane ring), 4.32 (s, 2H, NH_2, exchangeable with D_2O), 7.40–8.02 (m, 8H, aromatic-H) and 9.50, 10.00 (2s, 2H, 2NH,exchangeable with D_2O).

*5.1.15. 4-[Spiro [(2H, 3H) quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-benzoic acid [1-substituted aryl meth-(e)-ylidene]-hydrazides (**XVa**,**b**)*

Generalmethod: a mixture of compound **XIV** (3.50 g; 10 mmol) and the appropriate aldehyde, namely: *p*-methoxybenzaldehyde and 2-thiophenecarboxaldehyde (10 mmol) in absolute ethanol (30 mL) in the presence of few drops of acetic acid, was refluxed for 12 h. The reaction mixture was cooled and the formed precipitate was filtered off and recrystallized from the proper solvent to obtain the desired Schiff,s bases **XVa**,**b**.

*5.1.15.1. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]–benzoic acid-[1-(4-methoxy phenyl) meth-(e)-ylidene]-hydrazide (**XVa**).* Crystallized from ethanol to give light brown crystals, mp 241–243 °C, yield (73%). Analysis for $C_{28}H_{28}N_4O_3$, M.wt. (468.66). Calced.: %C, 71.77; H, 6.02; N, 11.95. Found: 71.90; H, 6.30; N, 11.66. IR (KBr, cm^{-1}): 3315, 3296 (2NH), 1670, 1645 (2CO) and 1604 (C═N). ^1H NMR (DMSO-d_6,δ ppm): 1.89 (s, 10H, cyclohexane ring), 3.80 (s, 3H, OCH_3), 6.00(s, 1H, CH═N), 6.8–7.6(m,12H, aromatic-H) and 8.10, 10.20 (2s, 2H, 2NH, exchangeable with D_2O).

*5.1.15.2. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]benzoicacid-[1-(2-thienyl)meth-(e)-ylidene]-hydrazide (**XVb**).* Crystallized from ethanol to give reddish brown crystals, mp 240–242 °C, yield (79%). Analysis for $C_{25}H_{24}N_4O_2S$, M.wt. (444.57). Calced.: %C, 67.54; H, 5.44; N, 12.60; S, 7.21. Found, %C, 67.35; H, 5.60; N, 12.32; S, 6.93. IR (KBr, cm^{-1}): 3346, 3246 (2NH), 1669, 1660 (2CO) and 1592 (C═N). MS (*m*/*z*): [M^+ +1] 445 (13%) and 120 (100%).

*5.1.16. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-[4-oxo-2-(substituted aryl) thiazolidin-3-yl] benzamides (**XVIa**,**b**)*

General method: a mixture of compounds **(XVIa**,**b)** (5 mmol) and thioglycolic acid (0.35 ml; 5 mmol) in dry benzene (20 mL) was refluxed for 16 h. The excess solvent was evaporated under reduced pressure and the obtained residue was treated with petroleum ether (60–80). The solid product was filtered off and washed with petroleum ether (60–80) to obtain the desired products **XVIa**,**b** respectively.

*5.1.16.1. 4-[Spiro [(2H,3H)- quinazoline-2,1′- cyclohexan]-4-(1H)-one-3-yl]-N-[4-oxo-2-(4-methoxyphenyl) thiazolidin-3-yl] benzamide (**XVIa**).* Crystallized from isopropanol to give brown crystals, mp 95–101 °C, yield (70%). Analysis for $C_{30}H_{30}N_4O_4S$, M.wt. (542.67). Calced.: %C, 66.39; H, 5.57; N, 10.32; S, 5.90. Found: %C, 66.56; H, 5.32; N, 10.10; S, 6.10. IR (KBr, cm^{-1}): 3297, 3272 (2NH), 3041(CH, aromatic), 2968 (CH, alicyclic), 1684, 1680 (2CO, cyclic amide), 1655 (CONH) and 1601(C═N). ^1H NMR (DMSO-d_6, δ ppm): 2.1 (s, 10H, cyclohexane ring), 3.70 (s, 2H, CH_2, thiazolidinone ring), 3.85 (s, 3H, OCH_3), 5.90 (s, 1H, CH of thiazolidinone ring), 7.00–8.50 (m, 12H, aromatic-H) and 10.21,10.50 (2s, 2H, 2NH, exchangeable with D_2O).

*5.1.16.2. 4-[Spiro[(2H,3H)-quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-[4-oxo-2-(thienyl) thiazolidin-3-yl] benzamide (**XVIb**).* Crystallized from ethanol to give brownish red crystals, mp 285–287 °C, yield (65%). Analysis for $C_{27}H_{26}N_4O_3S_2$, M.wt. (518.67). Calced.: %C, 62.52; H, 5.05; N, 10.80; S, 12.36. Found: %C, 62.31; H, 5.26; N, 11.00; S, 12.20. IR (KBr, cm^{-1}): 3354, 3209 (2NH), 3052 (CH, aromatic), 2923(CH, alicyclic), 1700, 1684 (2CO, cyclic amides), 1657 (CONH) and 1603 (C═N). ^1H NMR (DMSO-d_6, δ ppm): 2.1 (s, 10H, cyclohexane ring), 3.71 (s, 2H, CH_2, thiazolidinone ring,), 6.11 (s, 1H, CH, thiazolidinone ring), 7.20–8.50 (m, 11H, aromatic-H) and 10.2, 11.5 (2s, 2H, 2NH, exchangeable with D_2O). MS (*m*/*z*): [M + − 2] 516 (8%) and 55 (100%).

*5.1.17. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-substituted amido (thioamido) benzoic acid hydrazides (**XVIIa**–**c**)*

General method: a mixture of compound **XIV** (3.50 g; 10 mmol), the appropriate iso/isothiocyanate, namely: cyclohexyl isocyanate, cyclohexyl isothiocyanate and/or phenyl isothiocyanate (10 mmol) and few drops of triethylamine in dry benzene (20 ml) was refluxed for 12 h. The solvent was evaporated under reduced pressure and the obtained solid was recrystallized from the proper solvent to give the compounds **XVIIa**–**c**, respectively.

*5.1.17.1. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-(cyclohexyl) amido benzoic acid hydrazide (**XVIIa**).* Crystallized from isopropanol to give white crystals, mp 146–148 °C, yield (80%). Analysis for $C_{27}H_{33}N_5O_3$, M.wt. (475.59). Calced.: %C, 68.18; H, 6.99; N, 14.72. Found: %C, 68.22; H, 6.70; N, 14.40. IR (KBr, cm^{-1}): 3392, 3328, 3294,3107(4NH), 3067 (CH-aromatic), 2931 (CH, alicyclic), 1679, 1660, 1657 (3CO). ^1H NMR (DMSO-d_6, δ ppm): 1.00–1.20 (m, 6H, cyclohexyl), 1.50–1.71 (m, 4H, cyclohexyl), 2.00 (s, 10H, cyclohexane ring), 3.84 (m, 1H, CH–N), 7.61–8.10 (m, 8H, aromatic-H), 4.21, 7.00, 9.95 and 10.12 (4s, 4H, 4NH, exchangeable with D_2O).

*5.1.17.2. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-(cyclohexyl) thioamido benzoic acid hydrazide (**XVIIb**).* Crystallized from isopropanol to give white crystals, mp 211–213 °C, yield (73%). Analysis for $C_{27}H_{33}N_5O_2S$, M.wt. (491.66). Calced.: %C, 65.96; H, 6.76; N, 14.24; S, 6.52. Found: %C, 65.73; H, 6.98; N, 13.98; S, 6.76. IR (KBr, cm^{-1}): 3390, 3380, 3370, 3281 (4NH), 3012 (CH-aromatic), 2929 (CH, alicyclic), 1687, 1647 (2CO) and 1180 (C═S). ^1H NMR (DMSO-d_6, δ ppm): 1.00–1.11 (m, 6H, cyclohexyl), 1.50–1.71 (m, 4H, cyclohexyl), 2.00(s, 10H, cyclohexane ring), 4.00 (m, 1H, CH–N), 7.02–7.85 (m, 8H, aromatic-H), 4.25, 9.10, 10.11 and 10.20 (4s, 4H, 4NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, δ ppm): 32–40 (10 CH_2, cyclohexyl and cyclohexane rings), 53.9 (CH–NH), 71.8 (spiro head C), 118–143 (aromatic carbons), 165.8 (CO–NH), 170.0 (CO, quinazoline ring) and 185.0 (C═S).

*5.1.17.3. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-phenylthioamido benzoic acid hydrazide (**XVIIc**).* Crystallized from ethanol to give light brown crystals, mp192–201 °C, yield (80%). Analysis for $C_{27}H_{27}N_5O_2S$, M.wt.(485.61). Calced.: %C, 66.79; H, 5.60; N, 14.42; S, 6.60. Found: %C, 66.99; H, 5.78; N, 14.38; S, 6.75. IR (KBr, cm^{-1}): 3388, 3370, 3339, 3787 (4NH), 3041 (CH, aromatic), 1668 (CO, quinazoline ring), 1632 (CONH) and 1188 (C═S). ^1H NMR (DMSO-d_6,δ ppm): 2.00 (s, 10H, cyclohexane ring), 7.00–8.00 (m, 13H, aromatic-H) and 9.6, 9.7, 10.2, 10.4 (4s, 4H, 4NH, exchangeable with D_2O). MS (*m*/*z*): 485 (10%) and 162 (100%).

*5.1.18. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-[(2,4-dioxo/2-thio-4-oxo)-1-substituted-imidazolidin-3-yl]benzamides (**XVIIIa**–**c**)*

General method: a mixture of compound **XVIIa**–**c** (2 mmol), monochloroacetic acid (0.3 g; 3 mmol) and few drops of pyridine

were fused for 6 h. The obtained materiel was triturated with crushed ice, filtered off and crystallized from the proper solvent to obtain the imidazoline derivatives **XVIIIa–c**, respectively.

*5.1.18.1. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3yl]-N-(2,4-dioxo-1-cyclohexylimidazolidin-3-yl)benzamide (**XVIIIa**).* Crystallized from DMF/H_2O to give yellow crystals, mp129–131 °C, yield (65%). Analysis for $C_{29}H_{33}N_5O_4$, M.wt. (515.61). Calced.: %C, 67.55; H, 6.45; N, 13.58. Found: %C, 67.32; H, 6.44; N, 13.80. IR (KBr, cm^{-1}): 3349, 3340 (2NH), 2934 (CH, alicyclic), 1675, 1660 (2CO, lactamic). and 1640 (CO, amide). ^{1}H NMR (DMSO-d_6,δ ppm): 1.10–1.21 (m, 6H, cyclohexyl), 1.50–1.61 (m, 4H, cyclohexyl), 2.00 (s, 10H, cyclohexane ring), 3.91 (m, 1H, CH-N), 4.22 (s, 2H, CH_2, imidazolidine ring), 7.6–8.6 (m, 8H, aromatic-H) and 8.7–9.0 (2S, 2H, 2NH, exchangeable with D_2O). MS (*m*/*z*):M^+ 516 (5%) and 53(100%).

*5.1.18.2. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-(2-thio-4-oxo-1-cyclohexylimidazolidin-3-yl)benzamide (**XVIIIb**).* Crystallized from DMF/H_2O to give white crystals, mp 165–167 °C, yield (65%). Analysis for $C_{29}H_{33}N_5O_3S$, M.wt. (531.68). Calced.: %C, 65.51; H, 6.25; N, 13.17; S, 6.03. Found: %C, 65.26; H, 5.98; N, 13.00; S, 6.25.IR (KBr, cm^{-1}): 3259, 3247 (2NH), 3098(CH, aromatic), 2929(CH, alicyclic), 1675, 1672 (2CO, lactamic), 1641 (CO, amide) and 1181(C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.10–1.21 (m, 6H, cyclohexyl), 1.50–1.62 (m, 4H, cyclohexyl), 2.00 (s, 10H, cyclohexane ring), 4.11 (m, 1H, CH–N), 4.17 (s, 2H, CH_2, imidazolidine ring), 7.69–8.54 (m, 8H, aromatic-H) and 8.90–9.00 (2S, 2H, 2NH, exchangeable with D_2O). MS (*m*/*z*):[M^+ + 1] 532 (5%) and 53 (100%).

*5.1.18.3. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-(2-thio-4-oxo-1-phenylimidazolidin-3-yl) benzamide (**XVIIIc**).* Crystallized from DMF/H_2O to give brown crystals, mp 195–197 °C, yield (65%). Analysis for $C_{29}H_{27}N_5O_3S$, M.wt. (525.63). Calced.: %C, 66.26; H, 5.17; N, 13.32; S, 6.10. Found: %C, 66.54; H, 5.36; N, 13.00; S, 6.40. IR (KBr, cm^{-1}): 3360, 3281 (2NH), 3049 (CH, aromatic), 2987 (CH, alicyclic), 1670, 1665 (2CO, lactamic), 1645 (CO, amide) and 1183 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.00 (s, 10H, cyclohexane ring), 4.21 (s, 2H, CH_2 of imidazolidine ring), 7.51–8.20 (m, 13H, aromatic-H-) and 10.11, 11.13 (2S, 2H, 2NH, exchangeable with D_2O).

*5.1.19. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-[2,4,5,6-tetrahydro-(2,4,6-trioxo/2-thio-4,6-dioxo)-3-substituted pyrimidin-1-yl]benzamide (**XIXa–c**)*

General method: a mixture of compound **XVIIa–c** (2 mmol) and malonic acid (0.2 g; 2 mmol) in absolute ethanol (20 mL) was refluxed for 16h. The precipitated solid formed upon cooling, was filtered and recrystallized from the proper solvent to obtain the desired derivatives **XIXa–c**, respectively.

*5.1.19.1. 4-[Spiro [(2H, 3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-(2,4,5,6-tetrahydro-2,4,6-trioxo- 3-cyclohexyl pyrimidin-1-yl) benzamide (**XIXa**).* Crystallized from isopropanol to give white crystals, mp 235–237 °C, yield (70%). Analysis for $C_{30}H_{33}N_5O_5$, M.wt. (543.62). Calced.: %C, 66.28; H, 6.11; N, 12.88.Found: %C, 65.95; H, 6.38; N, 13.03. IR (KBr, cm^{-1}): 3319, 3251 (2NH), 3016 (CH, aromatic), 2930 (CH, alicyclic), 1694–1670 (4CO, lactamic) and 1641 (CO, amide). ^{1}H NMR (DMSO-d_6,δppm):1.00–1.21 (m, 6H, cyclohexyl), 1.50–1.71 (m, 4H, cyclohexyl), 2.00 (s,10H, cyclohexane ring), 3.62 (s, 2H, CH_2), 3.82 (m, 1H, CH–N), 7.61–8.11 (m, 8H, aromatic-H) and 9.99,10.11 (2s, 2H, 2NH, exchangeable with D_2O). MS (*m*/*z*): [M + − 1] 542 (5%) and 120 (100%).

*5.1.19.2. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-(2,4,5,6-tetrahydro- 2-thioxo-4,6-dioxo-3cyclohexyl pyrimidin-1-yl) benzamide (**XIXb**).* Crystallized from isopropanol to give white crystals, mp 128–130 °C, yield (60%). Analysis for $C_{30}H_{33}N_5O_4S$, M.wt. (559.69). Calced.: %C, 64.38; H, 5.94; N, 12.51; S, 5.72. Found, %C, 64.50; H, 5.73; N, 12.85; S, 5.45. IR (KBr, cm^{-1}): 3268, 3110 (2NH), 3055 (CH, aromatic), 2930 (CH, alicyclic), 1695–1670 (3CO lactamic), 1640 (CO, amide) and 1182 (C═S). ^{1}H NMR (DMSO-d_6, δ ppm): 1.00–1.21 (m, 6H, cyclohexyl), 1.51–1.81 (m, 4H, cyclohexyl), 2.00 (s, 10H, cyclohexane ring), 3.60 (s, 2H, CH_2), 4.11 (m, 1H, CH–N), 7.20–8.20 (m, 8H, aromatic-H) and 9.11, 10.20 (2s, 2H, 2NH, exchangeable with D_2O).

*5.1.19.3. 4-[Spiro[(2H,3H)quinazoline-2,1′-cyclohexan]-4-(1H)-one-3-yl]-N-(2,4,5,6-tetrahydro-2-thioxo-4,6-dioxo-3-phenyl pyrimidin-1-yl) benzamide (**XIXc**).* Crystallized from ethanol to give brownish yellow crystals, mp 112–114 °C, yield (73%). Analysis for $C_{30}H_{27}N_5O_4S$, M.wt. (553.64). Calced.: %C, 65.08; H, 4.91; N, 12.64; S, 5.79. Found: %C, 65.38; H, 4.77; N, 12.87; S, 5.88. IR (KBr, cm^{-1}): 3214, 3115 (2NH), 3046 (CH, aromatic), 2982 (CH, alicyclic), 1695-1670 (3CO, lactamic), 1640 (CO, amide) and 1181 (C═S). MS (*m*/*z*): [M^+ +1] 554 (15%), 73(100%).

5.2. Biological screening

5.2.1. Materials and methods

Animals-Adult rats of both sexes weighing 150–200 g and adult mice weighing 20–25 g were used in the experiments. Animals were housed under standardized conditions for light and temperature and received standard rat chow and tap water and libitum. Animals were randomly assigned to different experimental groups, each kept in a separate cage. All animal procedures were performed after approval from the Ethics committee of the National Research Center and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No.85-23, revised 1985).

5.2.2. Drugs and chemicals

Carrageenan Iambda Sigma–Aldrich chemical company (USA), indomethacin Khahira Pharmaceutical and Chemical Company (Cairo, Egypt) and tramadol October Pharma (Cairo, Egypt).

5.2.3. Antiinflammatory testing

The carrageenan rat paw oedema model of inflammation was used to evaluate the anti-inflammatory properties of the tested compounds. Rats were randomly assigned to treatment groups and sterile carrageenan lambda (100 ul of a 1% solution in saline) was injected sub-planter into right hind paw of the rat. Carrageenan caused visible redness and pronounced swelling that was well developed by 4 h and persisted for more than 48 h. Right hind paw was measured with a planimeter [53,54] before, and at 1,2,3 and 4 h after carrageenan injection. Due to water insolubility of the tested compounds, they were dissolved in DMSO then injected i.p (9 mg/kg b wt) [37].The control animals were injected (i.p) with appropriate volume of DMSO. The standard drug was indomethacin (10 mg/kg b wt). Different compounds or indomethacin were given 1hr before carrageenan injection.

5.2.4. Analgesia testing

The hot-plate test was performed on mice by using an electronically controlled hot-plate (ugo Basile, Italy) heated to 52 °C (±0.1 °C), for possible centrally mediated analgesic effect of the drugs.

Nineteen groups of rats each were given vehicle and/or the different compounds and the last group received tramadol (20 mg/

Kg b wt) 60 min prior to testing. Latency to lick a hind paw or jumping [55] was recorded sequentially before and at 1, 2 h post treatment.

5.2.5. Ulcerogenic effects

Groups of 5 male Wistar rats with a weight between 150 and 175 g are used. They are starved 48 h prior to drug administration. The test compounds are administered orally in 10 mL/kg as aqueous suspension. Doses are chosen which are highly active in the activity (9 mg/kg) and used. The animals are sacrificed after 7 h. Stomachs are removed and placed on saline soaked filter paper until inspection. A longitudinal incision along the greater curvature is made with fine scissor. The stomach is inverted over the index finger and the presence or the absence of gastric irritation is determined. The presence of a single or multiple lesions (erosion, ulcer or perforation) is considered to be positive [49]. The number of ulcers and the occurrence of hyperemia is noted (determine ulcer index).

5.3. Molecular modeling

All computational data were performed on Intel (R) Core (TM) 2 DUO CPU, 2.6 GHZ, 2.27 GHZ. With memory (RAM) = 4.00 GB and has Ubunto 8.04 release software. Docking was done by Plants software [50]and autodock vina [51,52].

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