

THE STRUCTURAL OPTIMIZATION OF [(2-R-QUINAZOLIN-4-YLIDENE)HYDRAZONO]CARBOXYLIC ACIDS AND ESTERS – APPROACH FOR CREATING A NEW CLASS OF COMPOUNDS WITH THE ANTICANCER ACTIVITY

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Key words: quinazolines; structural optimization; anticancer; structure – activity relationship

The strategies of the search of novel effective anticancer agents via optimization of [(2-R-quinazoline-4-ylidene)hydrazono]carboxylic acids and their esters are summarized in this article. To develop the most efficient synthetic approaches of initial substances the available information about chemical properties of [(2-R-quinazoline-4-ylidene)hydrazono]carboxylic acids and their derivatives has been generalized and systematized. The basic approaches for optimization of the initial substances structure included variation of substituents in position 2, creation and removal of conformational constraints and introduction of substituents into the cyclisation products. It has been shown that (3H-quinazoline-4-ylidene)hydrazine easily interacts with α , β , γ -ketocarboxylic acids and their esters with formation of the corresponding hydrazone derivatives. The given compounds are multicenter reagents, which are able to cyclize into the corresponding 3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones. 3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones are a class of electron-deficient heterocyclic systems and may be cleaved under the action of strong nucleophiles, in particular hydrazine, followed by formation of the corresponding 3-(2-aminophenyl)-6-R-1,2,4-triazine-5(2H)-ones or 3'-(2-aminophenyl)-3-R-spiro[piazoline-5,6'(1'H)-1,2,4-triazine]-5'(4'H)-ones. A comparative description of spectral characteristics of the compounds synthesized has also been presented. The highest anticancer activities have been shown by compounds 2c, 3b and 3c against leukemia cell lines CCRF-CEM ($\log GI_{50} = -6.10$; 6.05 ; 5.81 , respectively), compound 3b and 3c against breast cancer cell lines HS 578T ($\log GI_{50} = -5.83$; -6.43 , respectively). The results of SAR-analysis have been discussed and directions of further modification of the structures studied have been proposed.

СТРУКТУРНА ОПТИМІЗАЦІЯ [(2-R-ХІНАЗОЛІН-4-ІЛІДЕН)ГІДРАЗОНО]КАРБОНОВИХ КИСЛОТ ТА ЕСТЕРІВ – ПІДХІД ДО СТВОРЕННЯ НОВОГО КЛАСУ СПОЛУК З ПРОТИРАКОВОЮ ДІЄЮ

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Ключові слова: хіназоліни; структурна оптимізація; протираковий; взаємозв'язок структура – активність

Узагальнені стратегії пошуку нових ефективних протиракових агентів, засновані на структурній оптимізації [(2-R-хіназолін-4-іліден)гідразон]карбонових кислот та їх естерів. Для розробки найбільш ефективних синтетичних напрямків модифікації вихідних сполук проаналізовано та систематизовано наявний матеріал щодо хімічних властивостей [(2-R-хіназолін-4-іліден)гідразон]карбонових кислот та їх похідних. Основні напрямки оптимізації структури вихідних сполук полягали у варіюванні замісників у положенні 2, накладанні та усуненні конформаційних обмежень та введенні замісників у продукти їх циклізації. Показано, що 3Н-хіназолін-4-іліденгідразин легко взаємодіє з α , β , γ -кетокарбоновими кислотами та їх естераами з утворенням відповідних гідразонопохідних. Зазначені сполуки являють собою мультицентріві реагенти та здатні до циклізації у відповідні 3-R-2H-[1,2,4]триазино[2,3-c]хіназолін-2-они. 3-R-2H-[1,2,4]триазино[2,3-c]хіназолін-2-они являють собою електронодефіцитну гетероциклічну систему та розщеплюються під дією сильних нуклеофілів, зокрема гідразину, з утворенням відповідних 3-(2-амінофеніл)-6-R-1,2,4-триазин-5(2H)-онів або 3'-(2-амінофеніл)-3-R-спіро[піразолін-5,6'(1'H)-1,2,4-триазин]-5'(4'H)-онів. Представлено порівняльний опис спектральних характеристик одержаних речовин. Найбільшу протиракову активність показали сполуки 2c, 3b та 3c по відношенню до клітин лейкемії лінії CCRF-CEM ($\log GI_{50} = -6.10$; 6.05 ; 5.81 відповідно), сполуки 3b та 3c – раку молочної залози HS 578T ($\log GI_{50} = -5.83$; -6.43 відповідно). Обговорені результати SAR-аналізу та запропоновані перспективні напрямки модифікації описаних сполук з метою створення високоефективних протиракових агентів.

СТРУКТУРНА ОПТИМИЗАЦИЯ [(2-R-ХИНАЗОЛИН-4-ИЛИДЕН)ГИДРАЗОНО]КАРБОНОВЫХ КИСЛОТ И ЭФИРОВ – ПОДХОД К СОЗДАНИЮ НОВОГО КЛАССА СОЕДИНЕНИЙ С ПРОТИВОРАКОВОЙ АКТИВНОСТЬЮ

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Ключевые слова: хиназолины; структурная оптимизация; противораковый; взаимосвязь структура – активность

Обобщены стратегии поиска новых эффективных противораковых агентов, которые основаны на структурной оптимизации [(2-R-хиназолин-4-илiden)гидразон]карбоновых кислот и их эфиров. Для разработки наиболее эффективных синтетических направлений модификации исходных веществ проанализирован и систематизирован имеющийся материал относительно химических свойств [(2-R-хиназолин-4-илiden)гидразон]карбоновых кислот и их эфиров. Для разработки наиболее эффективных синтетических направлений модификации исходных веществ проанализирован и систематизирован имеющийся материал относительно химических свойств [(2-R-хиназолин-4-илiden)гидразон]карбоновых кислот и их эфиров.

назолин-4-илиден)гидразоно]карбоновых кислот и их производных. Основные направления оптимизации структуры исходных веществ заключались в варьировании природы заместителя в положении 2, наложении и снятии конформационных ограничений и введении заместителей в продукты их циклизации. Показано, что 3Н-хиназолин-4-илиден)гидразин легко взаимодействует с α, β, γ-кетокарбоновыми кислотами и их эфирами с образованием соответствующих гидразонопроизводных. Данные соединения представляют собой мультицентровые реагенты и способны циклизоваться в соответствующие 3-R-2H-[1,2,4]триазино[2,3-с]хиназолин-2-оны. 3-R-2H-[1,2,4]триазино[2,3-с]хиназолин-2-оны представляют собой электронодефицитные системы и расщепляются под действием сильных нуклеофилов, в частности гидразина, с образованием соответствующих 3-(2-аминофенил)-6-R-1,2,4-триазин-5(2Н)-онов или 3'-(2-аминофенил)-3-R-спиро[пиразолин-5,6'(1Н)-1,2,4-триазин]-5'(4Н)-онов. Представлено сравнительное описание спектральных характеристик полученных веществ. Наибольшую противораковую активность показали соединения 2с, 3в та 3с по отношению к клеткам лейкемии CCRF-CEM ($\log GI_{50} = -6.10; 6.05; 5.81$ соответственно), соединения 3в и 3с – рака молочной железы HS 578T ($\log GI_{50} = -5.83; -6.43$ соответственно). Обобщены результаты SAR-анализа и предложены перспективные направления модификации описанных соединений с целью создания высокоеффективных противораковых агентов.

Quinazoline derivatives have always attracted attention as a promising object of investigations aimed at designing novel effective medicines. This fact may be explained by a high biological activity of natural and synthetic quinazolines and wide possibilities of chemical modification of the given heterocyclic system. During last twenty years the interest to the class of compounds mentioned above has significantly increased as a result of revealing the anticancer activity among substituted 4-anilinoquinazolines [5, 6, 15, 18]. Now members of the series mentioned are well known as a reversible EGFR-mediated tyrosine kinase inhibitor and widely used in the treatment of non-small cell lung cancer, pancreatic cancer and some other oncology diseases. Obviously the medicines presented, as well as other anticancer drugs are far from perfect, there are a lot of side effects, contraindications and administration details that constrain their application in therapeutic regimens, therefore, the further design of quinazoline based anticancer agents with improved characteristics is of significant interest.

It is known that the search of novel bioactive agents among structural analogues of the existing drugs is a commonly used approach. Thus, we decided to search a new class of substances with the anticancer activity among 4-substituted quinazolines, namely [(2-R-quinazolin-4-ylidene)hydrazone]carboxylic acids and derived products. Our choice is based on the suppo-

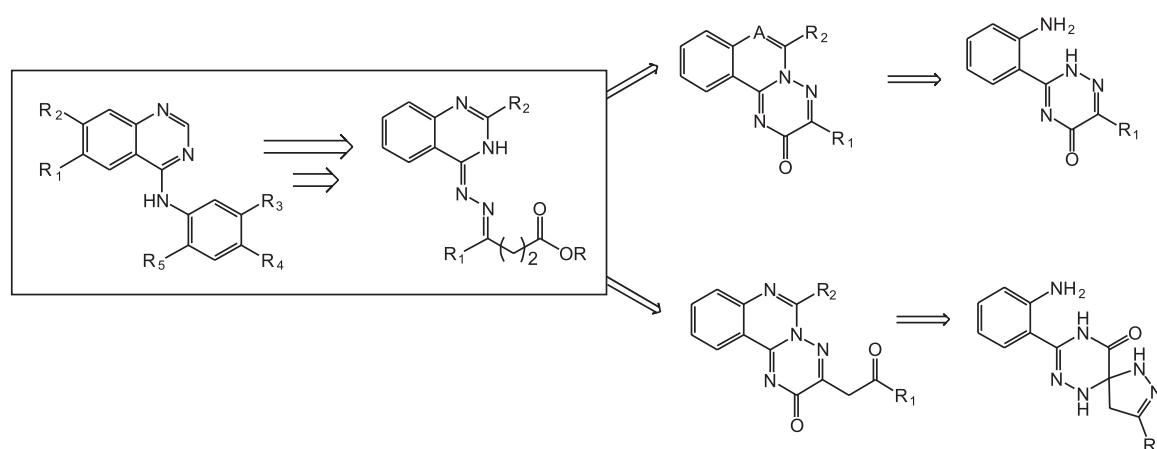
sition that combination of hydrazones of alkyl-(aryl-hetaryl-)carboxylic acids with the quinazoline fragment in one molecule, especially taken into account the broad antitumour potential of the latter, [1, 2, 12, 13] is a promising approach of finding new biologically active compounds.

Thus, the aim of this work is the purposeful search of anticancer agents in the range of [(2-R-quinazolin-4-ylidene)hydrazone]carboxylic acids based on the principles of the structural optimization of the molecule (Scheme 1) for detailed elaboration of the database and correlation of the "structure – antitumor activity".

Results and Discussion

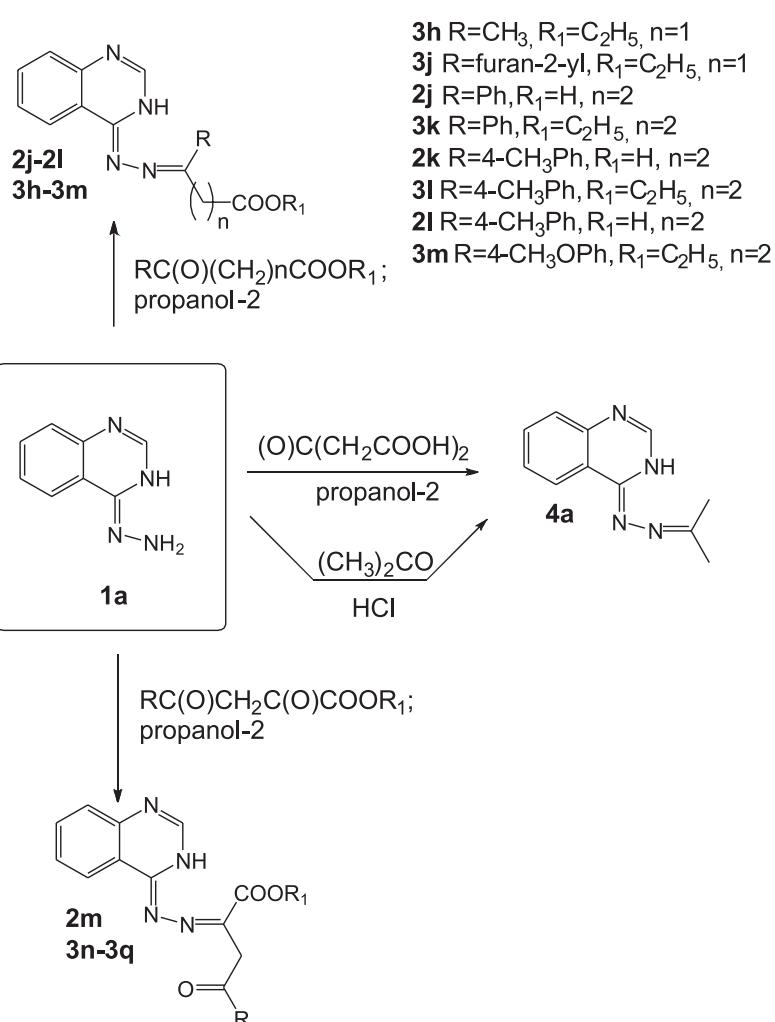
Chemistry

At the first stage [(2-R-3H-quinazolin-4-ylidene)hydrazone]carboxylic acids and their esters (**2a-2i**, **3a-3g**, Fig.) were synthesized by the interaction of (3H-quinazolin-4-ylidene)hydrazine (**1.1**) with α-oxocarboxylic acids and their esters in propan-2-ol with short-term heating or at room temperature. Similarly, the reaction of compound **1.1** with γ-oxocarboxylic acids and their esters, namely the formation of hydrazones **2j-2l**, **3h-3m**, occurs (Scheme 2). Whereas, to obtain the corresponding acid, in case of the failure with β-ketoglutaric acid, regardless of the temperature conditions of the reaction (transient heating,



Scheme 1. Structures of quinazoline-based compounds and their medicinal chemistry optimization.

- 2a** R=CH₃, R₁=H; **3a** R=CH₃, R₁=C₂H₅;
2b R=CH₂CH₂COOH, R₁=H
2c R=Bn, R₁=H; **3b** R=Bn, R₁=C₂H₅
2d R=4-NO₂Bn, R₁=H; **3c** R=4-NO₂Bn, R₁=C₂H₅
2e R=Ph, R₁=H; **3d** R=Ph, R₁=C₂H₅
2f R=4-CH₃Ph, R₁=H; **3e** R=4-CH₃Ph, R₁=C₂H₅
2g R=4-CH₃OPh, R₁=H; **3e** R=4-CH₃OPh, R₁=C₂H₅
2h =1,3,5-trimethylpyrazol-4-yl, R₁=H;
2i =thiophen-2-yl, R₁=H; **3g** thiophen-2-yl, R₁=C₂H₅



Scheme 2. Interaction of (3*H*-quinazolin-4-ylidene)hydrazine (**1a**) with oxocarboxylic and 2,4-diooxocarboxylic acids and their esters.

room or 0–10°C temperature) and the reaction medium (ethanol, ethanol – water (1:1), propan-2-ol) *N*-Isopropylidene-*N'*-(quinazolin-4(3*H*)-ylidene)hydrazine was the product of the reaction (**4a**, Scheme 2).

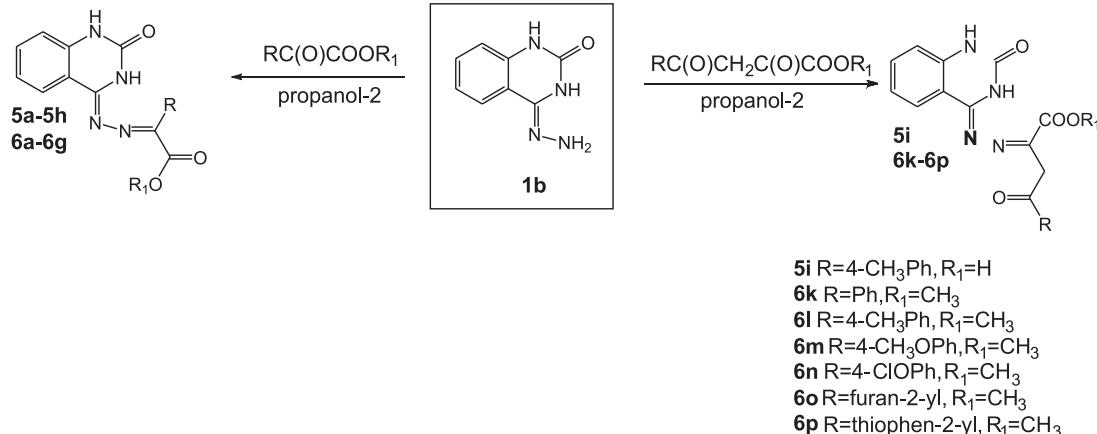
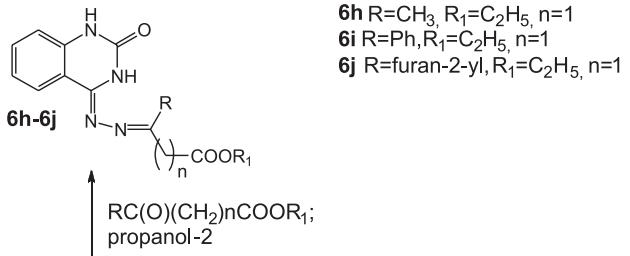
Under the given conditions hydrazine **1a** reacts with 4-aryl-(hetaryl)-2,4-dioxobutanoic acids and their esters on the carbonyl group at C(2) atom, with the formation of the corresponding hydrazones (**2m**, **3n**–**3q**). It is important to note that these reactions should be carried out at room temperature because in more severe conditions a mixture of products is formed: the corresponding hydrazones **3n**–**3q** and products of their heterocyclization – 3-[2-(het)aryl-2-oxoethyl]-2H-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones (**8a**–**8d**).

The quinazoline moiety was replaced by the quinazoline-2-one cycle in order to enhance the antitumour activity of the compounds synthesized and guided by the principles of the molecule optimization. The abovementioned reconstruction of the molecule estimates involving all functional groups, which are contacting with the biological target, in the initial state, and at the same time it leads to a new polar molecule fragment that can participate in the formation of hydrogen with the active conformation of the protein.

It is important that the reactivity of 4-hydrazono-1*H*-quinazolin-2-one (**1b**) with α -, β - and γ -oxocarboxylic acids and their esters in alcohols was slightly reduced and required acid catalysis. The low reactivity of compound **1b** was apparently due to the decrease of nucleophilic properties of the hydrazine group caused by the electron-electron withdrawing effect of the carbonyl group at position 2. Appropriate 2-R-[(2-oxo-2,3-dihydro-1*H*-quinazolin-4-ylidene)hydrazono]carboxylic acids and their esters were the products of the reaction (**5a**–**5h**, **6a**–**6j**, Scheme 3). Interaction of compound **1b** with 4-aryl-(hetaryl)-2,4-dioxobutanoic acids and their esters occurred in more severe conditions (DMF, acetic acid, 1,4-dioxane and the reaction time 1–2 h) and regardless of the solvent used the corresponding hydrazones were formed (**5i**, **6k**–**6p**, Scheme 3).

Conformational constraints were selected as the next step of the structural optimization implemented by one of the most common ways, namely cyclization, allowing to transform open side chains into cyclic fragments. The abovementioned modification explicitly led to significant changes in the spatial reorganization of the molecule. Therefore, it was logical to study cyclocondensation reactions of [(quinazo-

5a R=CH₃, R₁=H; **6a** R=CH₃, R₁=C₂H₅;
5b R=CH₂CH₂COOH, R₁=H
6b R=Bn, R₁=C₂H₅
5c R=4-NO₂Bn, R₁=H; **6c** R=4-NO₂Bn, R₁=C₂H₅
5d R=Ph, R₁=H; **6d** R=Ph, R₁=C₂H₅
5e R=4-CH₃Ph, R₁=H; **6e** R=4-CH₃Ph, R₁=C₂H₅
5f R=4-CH₃OPh, R₁=H; **6f** R=4-CH₃OPh, R₁=C₂H₅
5g=1,3,5-trimethylpyrazol-4-yl, R₁=H;
5h=thiophen-2-yl, R₁=H; **6g** thiophen-2-yl, R₁=C₂H₅



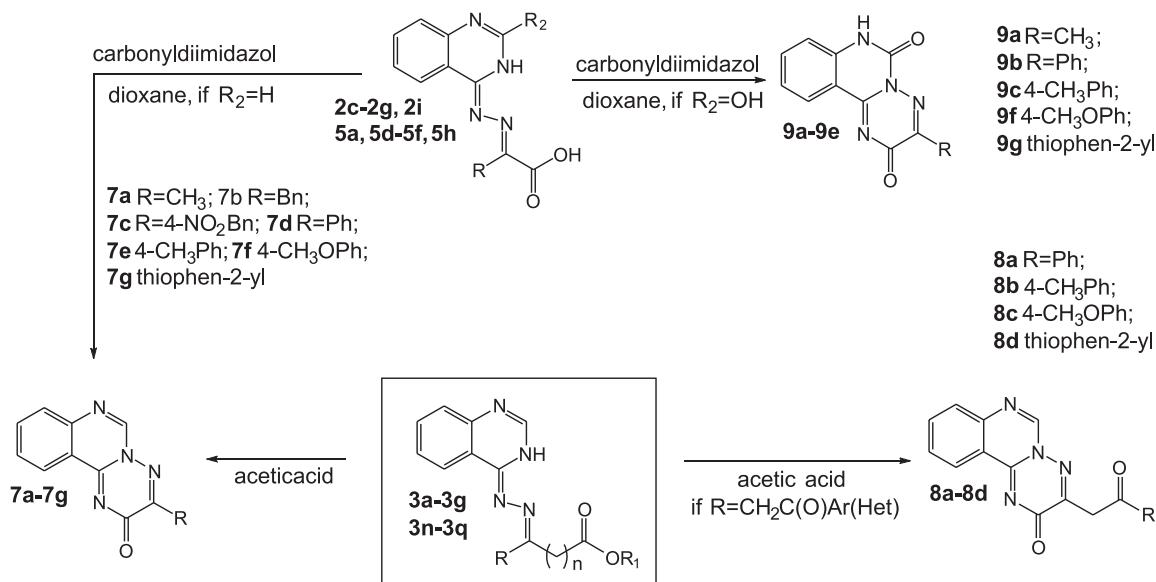
Scheme 3. Interaction of 4-hydrazono-1*H*-quinazolin-2-one (**1b**) with oxocarboxylic and 2,4-dioxocarboxylic acids and their esters.

lin-4(3*H*)-ylidene)hydrazone]carboxylic acids (**2a-2m**), esters (**3a-3q**) and [(2-oxo-2,3-dihydroquinazolin-4(1*H*)-ylidene)hydrazone]carboxylic acids (**5a-5i**), esters (**6a-6p**) for their further study as potential anticancer agents.

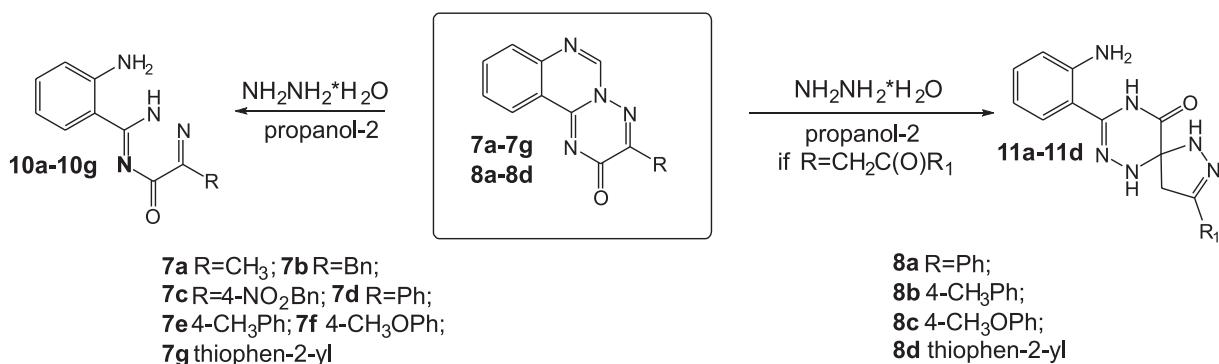
Esters **3a-3g**, **3n-3q** quite easily gave cyclocondensation in the medium of glacial acetic acid with formation of 3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2,6(7*H*)-diones (**7a-7g**) and 3-[2-(het)aryl-2-oxoethyl]-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-2-ones (**8a-8d**), respectively (Scheme 4) [7, 8]. In the case of esters **6a-6p**, cyclocondensation was impossible due to re-

duction of nucleophilicity of the quinazoline N₃-atom caused by the electron-electron withdrawing effect of the neighbouring carbonyl group. Thus, we used a different strategy, namely the cyclocondensation of acids (**5a**, **5d-5f**, **5h**, **2c-2g**, **2i**) in the corresponding 3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2,6(7*H*)-diones (**9a-9e**) and 3-R-2*H*-[1,2,4]triazino[2,3-*c*]quinazoline-2-ones (**7a-7g**), via activating the carboxyl group with CDI in anhydrous dioxane or DMF (Scheme 4) [11, 16].

Following the above strategy of purposeful search of anticancer agents, we made further changes in the



Scheme 4. Cyclocondensation of [(quinazolin-4(3*H*)-ylidene)hydrazone]carboxylic acids (**2c-2g**, **2i**), esters (**3a-3g**, **3n-3q**) and [(2-oxo-2,3-dihydroquinazolin-4(1*H*)-ylidene)hydrazone]carboxylic acids (**5a**, **5d-5f**, **5h**).



Scheme 5. Nucleophilic degradation of 3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones (**7a-7g**) and 3-[2-(het)aryl-2-oxoethyl]-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (**8a-8d**).

molecule conformation of 3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones (**7a-7g**) and 3-[2-(het)aryl-2-oxoethyl]-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (**8a-8d**). To solve this problem the well known methods [17], namely nucleophilic degradation of compounds **7a-7g**, **8a-8d** by hydrazine hydrate in propan-2-ol (Scheme 5), were used. The corresponding 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2H)-ones (**10a-10e**) were obtained as a result of interaction of compounds **7a-7g** with the given nucleophile, while compounds **8a-8d** formed 3'-(2-aminophenyl)-3-R-spiro[pyrazoline-5,6'(1'H)-1,2,4-triazin]-5'(4'H)-ones (**11a-11d**) [7]. It should be also noted that for the corresponding 3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2,6(7H)-diones (**9a-9e**) the reaction indicated failed.

The structure and purity of the compounds synthesized were confirmed by elemental analysis, ¹H and ¹³C NMR, LS-MS and EI-MS data. Chromatography-mass spectrometry studies of compounds (**2a-2m**, **3a-3q**, **4a**, **5a-5i**, **6a-6p**, **7a-7g**, **8a-8d**, **9a-9e**, **10a-10g**, **11a-11d**) in the “soft” ionization (APCI) allowed to register in each case the peak of a quasi-molecular ion [$M+1$], which had a high intensity and clearly demonstrated the individuality of the compounds synthesized.

According to the ¹H-NMR spectra a broad singlet of NH-proton at low-field at 13.84-11.43 ppm and a set of signals of quinazoline protons were characteristic for compounds **2a-2m** and **3a-3q**; it allowed to describe the substances studied as 3*H*-tautomeric forms. It is important that enhydrazine-hydrazone tautomerism was characteristic for compounds **3a-3c**, **3h-3j** in DMSO-d₆. Thus, compound **3i** had signals of =NNH- and =CH-groups at 12.13 and 4.28 ppm, respectively, indicating its existence in the enhydrazine form. At the same time ¹H NMR spectra of compounds **3a-3c**, **3j** were characterized by a distinct singlet of CH₂ signals at 3.96 and 3.94 ppm, respectively, suggesting the existence of the only hydrazone form of these compounds. Signals of enhydrazine and hydrazone forms in spectra of compound **3h** in the ratio (1:1) were registered. Interestingly that compounds **3n-3q** in DMSO-d₆ existed as hydrazones, but with

the characteristic different type of tautomerism – keto-enol one. Thus, ¹H NMR spectra of compounds **3n-3q** were characterized by a one-proton singlet of the =CH-group for the enol form at 5.96-5.88 ppm and a two-proton singlet of -CH₂-groups for the keto form at 4.56-4.55 ppm. In addition to these signals characterizing this tautomerism one could observe a one-proton singlet at low-field of the =C-OH group at 13.72-13.44 ppm, doubled singlets of the imide proton (3-NH) and doubled singlets of aliphatic protons (the ratio of tautomers (1:1).

Broad singlets in the low-field spectrum of two-NH protons at 11.07-10.76 ppm (N-1) and 10.72-9.49 ppm (N-3) were observed for compounds **5a-5i**, **6a-6p** characterizing these compounds as 1*H*, 3*H*-tautomers. In addition, a diamagnetic shift of aromatic protons of the quinazoline cycle through the screening effect of the ureide moiety was observed for 2-oxoderivatives **5a-5i**, **6a-6p**. It is important that compounds **5a-5i**, **6a-6p** exist as hydrazones, and only in case of compound **6h** doubled signals of CH/CH₂- and =N/NH-groups have been recorded, indicating the enhydrazine-hydrazone tautomerism. It is interesting that introducing into the second position the acceptor group (oxygen) affects the keto-enol tautomerism of compounds **6k-6p** in such a way that they exist only as keto derivatives.

¹H NMR spectra of compounds **7a-7h** obtained by alternative methods had a characteristic singlet of H-6 proton at 9.09-8.80 ppm and a doublet of H-11 at 8.67-8.52 ppm. It clearly confirms their structure and significantly differ them from compounds **3a-3f**. ¹H NMR spectra of compounds **8a-8d** had a similar pattern and were characterized by a singlet of proton H-6 in the region of 8.99-8.98 ppm and by a one-proton doublet of H-11 at 8.55-8.59 ppm, whereas compounds **9a-9e** had a one-proton doublet of H-11 due to the deshielding effect of the ureide fragment, which resonated in a weaker field at 8.29-8.27 ppm. Additionally, the compounds synthesized were characterized by a one-proton singlet of the phenacyl CH₂ group at 4.58-4.52 ppm (compounds **8a-8d**), by a broad singlet of the NH-group at 12.43-11.97 ppm

(compounds **9a-9e**). The ^{13}C NMR spectra were also in good agreement with the assigned structures of **7a-7h**, **8a-8d** and **9a-9e**.

Comparing data of ^1H NMR-spectra of compounds **7a-7h** and **8a-8d** with compounds **10a-10f** and **11a-11d** it should be noted the disappearance of a low-intensive singlet of proton H-6 and the appearance of a two-proton signal of the NH_2 -group, which clearly indicated the pyrimidine ring degradation. The signals of the amino-group appeared as a broad two-proton singlet at 9.5-8.44 ppm (**10a-10g**) and 6.32-6.31 ppm for compounds **11a-11d**. The signal of NH-proton of triazine and pyrazoline systems for compounds **11a-11d** was detected in some cases as low intensive broad singlets. The diastereotopic hydrogens of the CH_2 -group of the pyrazoline ring (**11a-11d**) appeared as two distinct doublets with $J_{\text{HH}} = 17.6$ Hz in the range of 2.89-3.07 and 3.77-4.04 ppm.

Signals in ^{13}C NMR spectra correspond exactly to the proposed structure of the compounds synthesized **10a-10g**. Characteristic signals caused by the C5 atom of the triazine system were detected at 160.6-149.8 ppm. Signals of the spiro C-atom in ^{13}C NMR spectra of compounds **11a-11d** were registered at 77.84-77.58 ppm serving as a reliable evidence of the spiro-heterocycles formation. The imine carbon C(3) of the pyrazoline ring was subjected to the influence of substituents in the het(aryl) ring and its signal was consequently predictably shifted in ^{13}C NMR spectra (148.26-147.58 ppm). The atom C(10) of the amide group was observed almost in the same region (163.45-164.08 ppm) as the corresponding one in the starting triazinoquinazolines.

The MS (EI) pattern is typical for this class of the heterocyclic system and is concerned mainly with the following processes. The basic direction of the molecular ions fragmentation of compounds **7d-7i** was associated with the fragmentation of the C(2)-C(3) and N(4)-N(5) and formation of fragments with m/z 171. The aryl(hetaryl)acyl moiety of compounds **8c** and **8d** was ruptured at the first stage, forming the characteristic $\text{ArC}\equiv\text{O}^+$ ions. The second stage was concerned with C(2)-C(3) and N(4)-N(5) bond breaking of triazinoquinazoline, which gave the fragment ion with m/z 171 (**8c**) or m/z 170 (**8d**). The mass spectrum of compound **9a** had a similar fragmentation of the molecular ion with compounds **7d-7h** forming the ion m/z 187 due to the rupture of the C(2)-C(3) and N(3)-N(4).

Evaluation of the anticancer activity *in vitro*

Newly synthesized compounds were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for the *in vitro* cell line screening to investigate their anticancer activity. Anticancer assays were performed according to the US NCI protocol described elsewhere [3, 4, 14]. Compounds were firstly evaluated at one

dose primary anticancer assay relative to approximately 60 cell lines (the concentration was 10 μM). The human tumour cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. In the screening protocol, each cell line was inoculated and preincubated for 24-48 h on a microtiter plate. Test agents were then added at a single concentration, and the culture was incubated for further 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each agent tested were reported as the percent growth of the treated cells comparing to the untreated control cells. The preliminary screening results are shown in Tab. 1.

The screening of the compounds synthesized in the concentration of 10 μM showed that individual cell lines had different sensitivity to the compounds synthesized. Thus, significant antitumour activity among the substances studied revealed compounds **2c**, **3b** and **3c**, which turned out to be active against most cancer cell lines (the mean growth, 62.92, 45.37, 47.94%, respectively). Among these compounds, ethyl 3-phenyl-2-[quinazolin-4(3*H*)-ylidene-hydrazono]propanoate (**3b**) possessed a high cytotoxic effect against cell lines (CCRF-CEM, K-562, MOLT-4, RPMI -8226) of leukemia and a proliferative effect against cell lines (HL-60 (TB), SR) of leukemia (Fig.). In addition, these compound **3b** had a wide spectrum of the antitumour activity in comparison with compounds **2c** and **3c** (Tab. 2).

According to the standard procedure the dose-dependent action in 5 concentrations of NCI at 59 cell lines of 9 types of cancer (100 μM -0.01 μM) was studied for compounds **2c**, **3b** and **3c**. Three dose-dependent parameters were calculated during our study: 1) GI_{50} – the molar concentration of the compound that inhibited 50% net cell growth; 2) TGI – the molar concentration of the compound leading to total inhibition of the cell growth; 3) LC_{50} – the molar concentration of the compound leading to 50% net cell death. If logarithmic data of the parameters studied ($\log \text{GI}_{50}$, $\log \text{TGI}$ and $\log \text{LC}_{50}$) were less than -4.00, compounds were marked as active.

Parameters of the compound activity against the most sensitive cell lines are shown in Table 2 ($\log \text{GI}_{50} \leq -5.60$). It is necessary to mention the selective activity of compounds **2c**, **3b** and **3c** against cell lines of leukemia (CCRF-CEM), NSC lung cancer (NCI-H226). So, substances **2c**, **3b** and **3c** revealed the high level of inhibition against cell line CCRF-CEM of leukemia ($\log \text{GI}_{50} = -6.10$; -6.05; -5.81, respectively). It is important that compounds **3b** and **3c** effectively inhibited the growth of cell lines HS 578T of breast cancer ($\log \text{GI}_{50} = -5.83$; -6.43, respectively).

It is important that among the substances under research compound **3.2** has a high anticancer acti-

Table 1

Anticancer screening data in the concentration of 10 µM

Compound	60 Cell lines assay in 1-dose 10µM conc.				Active (selected for 5-dose 60 cell lines assay)
	Mean growth %	Range of growth %	The most sensitive cell line ^a	Growth % of the most sensitive cell line	
2b	111.15	82.86 to 149.71	RPMI-8226/L	82.86	Inactive
2c	62.92	-24.95 to 123.65	SR/L	-24.95	Active
2e	97.84	-4.81 to 174.14	HS 578T/BC	-4.81	Inactive
2h	90.09	28.89 to 247.08	OVCAR-3/OV	28.89	Inactive
2i	100.48	53.43 to 154.20	RXF 393/RC	53.43	Inactive
2m	105.64	79.30 to 140.70	HOP-92/nsCLC	79.60	Inactive
3b	45.37	-30.08 to 85.34	SR/L	-30.08	Active
3c	47.94	-0.68 to 90.84	BT-549/BC	-0.68	Active
3f	102.05	66.50 to 135.41	786-0/RC	66.50	Inactive
3j	94.90	23.66 to 130.35	MDA-MB-468/BC	23.66	Inactive
3o	99.47	60.99 to 159.34	IGROV1/OV	60.99	Inactive
3p	105.67	48.38 to 208.57	SNB-75/CNSC	48.38	Inactive
3q	109.96	25.14 to 140.72	SR/L	25.14	Inactive
5h	101.98	16.80 to 138.20	UACC-257/M	16.80	Inactive
5i	107.02	45.11 to 131.70	HOP-62/nsCLC	45.11	Inactive
6j	102.20	21.49 to 157.22	MDA-MB-468/BC	21.49	Inactive
6l	110.69	-39.43 to 159.85	786-0/RC	-39.43	Inactive
6m	107.26	78.70 to 143.99	RPMI-8226/L	78.70	Inactive
6n	108.96	77.16 to 148.43	RPMI-8226/L	77.16	Inactive
7a	99.23	69.07 to 170.33	IGROV1/OV	69.07	Inactive
7b	95.04	10.25 to 16.54	NCI-H522/nsCLC	10.25	Inactive
7c	112.28	82.17 to 150.83	HL-60(TB)/L	82.17	Inactive
7d	93.73	-9.11 to 167.26	HS 578T/BC	-9.11	Inactive
7f	94.80	43.38 – 138.34	RXF 393/RC	43.38	Inactive
7g	92.99	41.66 – 134.16	IGROV1/OV	41.66	Inactive
8a	100.64	70.10 – 146.04	RXF 393/RC	70.10	Inactive
8c	102.44	68.90 – 163.27	IGROV1/OV	68.90	Inactive
9a	101.64	77.81 – 128.07	HOP-92/nsCLC	77.81	Inactive
9b	105.44	85.91 – 137.18	SNB-75/CNSC	85.91	Inactive
9c	103.30	61.69 – 138.32	EKXV/nsCLC)	61.69	Inactive
9d	105.10	64.32 – 183.56	HL-60(TB)/L	64.32	Inactive
9e	106.53	73.83 – 298.40	HL-60(TB)/L	73.83	Inactive
10a	100.06	67.17 – 125.28	RPMI-8226/L	67.17	Inactive
10b	96.27	69.88 – 115.45	RPMI-8226/L	69.88	Inactive
10c	109.03	69.48 – 143.11	HL-60(TB)/L	69.48	Inactive
10e	102.36	23.98 – 129.53	UACC-257/M	23.98	Inactive
10f	100.91	27.48 – 133.38	UACC-257/M	27.48	Inactive
10g	103.34	63.19 – 146.15	CAKI-1/RC	63.19	Inactive
11c	93.86	54.54 – 128.15	RPMI-8226/L	54.54	Inactive
11d	93.38	35.69 – 125.01	HCC-2998/ColC	35.68	Inactive

a – L – leukemia, nsCLC – non-small cell lung cancer, ColC – colon cancer, CNSC – CNS cancer, M – melanoma, OV – ovarian cancer, RC – renal cancer, PC – prostate cancer, BC – breast cancer.

Table 2

The effect of compounds 2c, 3b and 3c on the growth of individual tumour cell lines ($\log GI_{50} < -5.60$)

Compd	Disease	Cell line	$\log GI_{50}$	$\log TGI$	$\log LC_{50}$
2c	Leukemia	CCRF-CEM	-6.10	-4.48	>-4.00
		HL-60(TB)	-5.64	-5.11	>-4.00
	NSC lung cancer	NCI-H226	-5.64	-4.11	>-4.00
		OVCAR-3	-5.68	-5.08	>-4.00
	Prostate Cancer	DU-145	-5.86	>-4.00	>-4.00
3b	Leukemia	CCRF-CEM	-6.05	-5.06	>-4.00
		MOLT-4	-5.66	-4.15	>-4.00
		SR	-5.78	-4.47	>-4.00
	NSC lung cancer	NCI-H226	-5.61	>-4.00	>-4.00
		HCT-15	-5.77	>-4.00	>-4.00
	Ovarian Cancer	IGROV1	-5.70	>-4.00	>-4.00
	Renal cancer	UO-31	-5.83	>-4.00	>-4.00
3c	Breast Cancer	HS 578T	-5.83	-5.11	>-4.00
	Leukemia	CCRF-CEM	-5.81	>-4.00	>-4.00
	Breast Cancer	HS 578T	-6.43	-5.29	-4.26

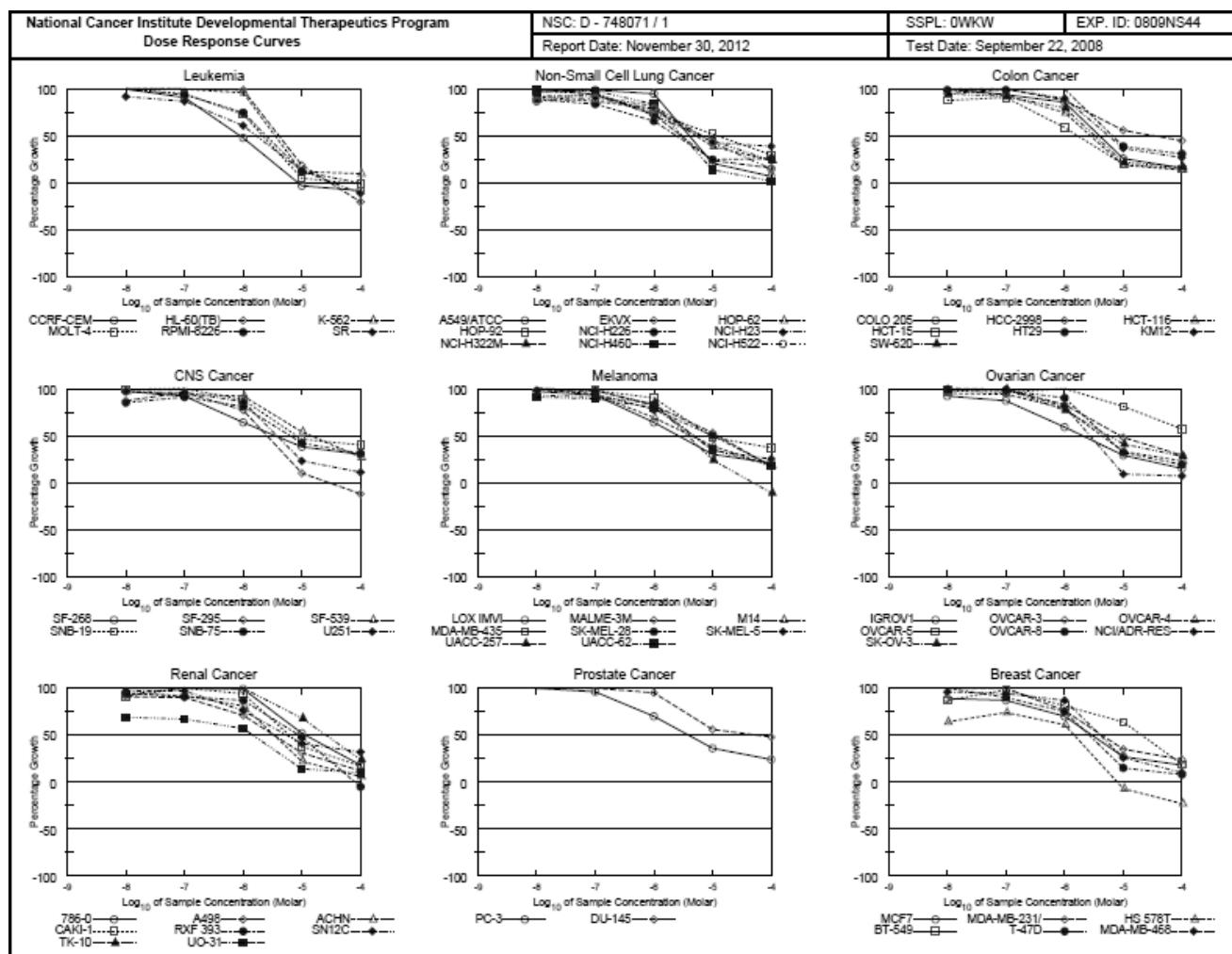


Fig. The effect of compound 3.2 on the growth of individual tumour cell lines.

Table 3

The anticancer selectivity pattern
(the average value of LogGI₅₀) of the most active
compounds 2c, 3b and 3c

Disease	Compound		
	2c	3b	3c
MGMID (N)*	-5.14 (59)	-5.30 (59)	-5.13 (59)
L	-5.49	-5.66	-5.29
nsCLC	-5.17	-5.31	-5.13
CoIC	-5.18	-5.30	-5.11
CNSC	-5.05	-5.27	-5.15
M	-5.06	-5.25	-4.99
OV	-5.23	-5.18	-5.04
RC	-5.03	-5.23	-5.05
PC	-4.26	-4.81	-4.97
BC	-5.27	-5.40	-5.36

* – N – is the number of sensitive cell lines against which the compound possessed a considerable growth inhibition according to the parameters log GI₅₀, log TGI and log LC₅₀ ≤ 4.00.

vity against cell lines of leukemia (the average data LogGI₅₀ = -5.66), NSC lung cancer (the average data LogGI₅₀ = -5.31), colon cancer (the average data LogGI₅₀ = -5.30) and breast cancer (the average data LogGI₅₀ = -5.40) (Tab. 3, Fig.).

SAR Discussion

The analysis of relationships between the chemical structure and the anticancer activity of the compounds synthesized showed that in most cases chemical modification aimed to the conformational constraints in molecules had a negative effect on the expression of the cytotoxic action. The transformation mentioned above allow changing significantly the sensitivity spectrum of compounds. However, the cyclization of [(2-R-3H-quinazolin-4-ylidene)hydrazone] carboxylic acids and their esters into the corresponding 3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones in some cases had no effect on the antitumour action against HS 578T/BC (breast cancer) cell line. It may be explained by a determinative role of the presence of the quinazoline fragment for this type of action. Nucleophilic cleavage of the pyrimidine ring of 3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2-ones also cause the change of the activity spectrum. Thus, the most sensitive to 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2H)-ones cell lines are RPMI-8226/L (Leukemia) and UACC-257/M (Melanoma).

Experimental Protocols

Materials and Methods

Melting points were determined in open capillary tubes and were uncorrected. The elemental analyses (C, H, N, S) were performed using an ELEMENTAR vario EL cube analyzer (USA). Analyses were indi-

cated by the symbols of the elements or functions within ±0.3% of the theoretical values. IR spectra (4000-600 cm⁻¹) were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Bioscience, Germany) using a module for measuring attenuated total reflection (ATR). ¹H NMR spectra (400 MHz) and ¹³C NMR spectra (100 MHz) were recorded on Varian-Mercury 400 (Varian Inc., Palo Alto, CA, USA) spectrometers with TMS as an internal standard in DMSO-d₆ solution. LC-MS were recorded using chromatography/mass spectrometric system consisting of an "Agilent 1100 Series" high performance liquid chromatograph (Agilent, Palo Alto, CA, USA) equipped with a diode-matrix and an "Agilent LC/MSD SL" mass-selective detector (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian, USA). The purity of all the compounds obtained was checked by ¹H-NMR and LC-MS.

Substances **1a** and **1b** were synthesized according to the reported procedures [10, 11]. Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

Chemistry

The general method of synthesis of [(quinazolin-4(3H)-ylidene)hydrazone]carboxylic acids (2a-2m).

Add oxocarboxylic acid (0.005 Mol) to the suspension of 0.8 g (0.005 Mol) of (quinazolin-4(3H)-ylidene)hydrazine (**1.1**) in 15 ml of propan-2-ol, boil the resulting mixture for 10-15 min (in case of γ-oxocarboxylic acid add 1 drop of hydrochloric acid and boil for 45-60 min; in case of 2,4-oxocarboxylic acids allow to stand at room temperature for 30 min). Cool the mixture and dilute with water. Filter the precipitate formed and dry.

2-[Quinazolin-4(3H)-ylidenehydrazone]propanoic acid (2a). Yield – 82.6%. M.p. – 206-208°C; ¹H-NMR, δ: 2.26 (s, 3H, CH₃), 7.53 (t, 1H, H-7), 7.62 (d, 1H, H-8), 7.78 (t, 1H, H-6), 8.13 (s, 1H, H-2), 8.33 (d, 1H, J=8.2 Hz, H-5), 12.00 (s, 1H, 3-NH); LC-MS, m/z=230 [M+1]; Anal. calcd. for C₁₁H₁₀N₄O₂: C, 57.39; H, 4.38; N, 24.34. Found: C, 57.38; H, 4.37; N, 24.38.

2-[Quinazolin-4(3H)-ylidenehydrazone]pentanedioic acid (2b). Yield – 87.7%. M.p. – 232-234°C; ¹H-NMR (400 MHz) δ: 2.12 (d, 4H, -(CH₂)₂-), 7.43 (t, 1H, H-7), 7.55 (d, 1H, H-8), 7.68 (t, 1H, H-6), 7.93 (s, 1H, H-2), 8.03 (d, 1H, H-5), 12.34 (s, 1H, 3-NH); LC-MS, m/z=289 [M+1]; Anal. calcd. for C₁₃H₁₂N₄O₄: C, 57.17; H, 4.20; N, 19.44; Found: C, 57.18; H, 4.17; N, 19.43.

3-Phenyl-2-[quinazolin-4(3H)-ylidenehydrazone]propanoic acid (2c). Yield – 78.4%. M.p. – 207-209°C; ¹H-NMR, δ: 4.18 (s, 2H, CH₂), 7.14 (t, 1H, J=7.0 Hz, H-4 Ph), 7.23 (t, 2H, J=7.4 Hz, H-3, H-5 Ph), 7.29 (d, 2H, J=7.4 Hz, H-2, H-6 Ph), 7.53 (t, 1H, J=7.4 Hz, H-7), 7.61 (d, 1H, J=8.2 Hz, H-8), 7.77 (t, 1H, J=7.8 Hz, H-6), 8.12 (s, 1H, H-2), 8.38 (d, 1H, J=8.0 Hz, H-5), 12.35

(s, 1H, 3-NH); LC-MS, m/z =307 [M+1]; Anal. calcd. for $C_{17}H_{14}N_4O_2$: C, 66.66; H, 4.61; N, 18.29. Found: C, 66.68; H, 4.63; N, 18.31.

3-(4-Nitrophenyl)-2-[quinazolin-4(3H)-ylidenehydrazone]propanoic acid (2d**)**. Yield – 85.5%. M.p. – 196-200°C; 1 H-NMR, δ : 4.33 (s, 2H, CH_2), 7.56 (m, 3H, H-7 Quin, H-2, H-6 Ph), 7.64 (d, 1H, J =8.2 Hz, H-8), 7.78 (t, 1H, J =8.0 Hz, H-6), 8.10 (d, 2H, J =7.6 Hz, H-3, H-5 Ph), 8.17 (s, 1H, H-2), 8.36 (d, 1H, J =7.8 Hz, H-5); LC-MS, m/z =352 [M+1]; Anal. calcd. for $C_{17}H_{13}N_5O_4$: C, 58.12; H, 3.73; N, 19.93. Found: C, 58.14; H, 3.75; N, 19.91.

Phenyl-[quinazolin-4(3H)-ylidenehydrazone]acetic acid (2e**)**. Yield – 92.5%. M.p. – 208-210°C; 1 H-NMR, δ : 7.45-7.35 (m, 4H, H-7 Quin, H-3, H-4, H-5 Ph), 7.50 (d, 1H, J =8.2 Hz, H-8), 7.63 (t, 1H, J =6.8 Hz, H-6), 7.85 (s, 1H, H-2), 7.95-7.90 (m, 2H, H-2, H-6 Ph), 8.23 (d, 1H, J =7.6 Hz, H-5), 12.26 (s, 1H, 3-NH); LC-MS, m/z =293 [M+1]; Anal. calcd. for $C_{16}H_{12}N_4O_2$: C, 65.75; H, 4.14; N, 19.17. Found: C, 65.74; H, 4.15; N, 19.19.

(4-Methylphenyl)-[quinazolin-4(3H)-ylidenehydrazone]acetic acid (2f**)**. Yield – 88.1%. M.p. – 194-196°C; 1 H-NMR, δ : 2.38 (s, 3H, CH_3), 7.34 (d, 2H, J =7.9 Hz, H-3, H-5 Ph), 7.47 (t, 1H, J =7.7 Hz, H-7), 7.55 (d, 1H, J =7.7 Hz, H-8), 7.71 (t, 1H, J =7.5 Hz, H-6), 7.84 (d, 2H, J =8.1 Hz, H-2, H-6 Ph), 7.93 (s, 1H, H-2), 8.13 (d, 1H, J =7.7 Hz, H-5), 11.83 (s, 1H, 3-NH); LC-MS, m/z =307 [M+1]; Anal. calcd. for $C_{17}H_{14}N_4O_2$: C, 66.66; H, 4.61; N, 18.29. Found: C, 66.67; H, 4.59; N, 18.27.

(4-Methoxyphenyl)-[quinazolin-4(3H)-ylidenehydrazone]acetic acid (2g**)**. Yield – 86.8%. M.p. – 184-186°C; 1 H-NMR, δ : 3.85 (s, 3H, OCH_3), 7.08 (d, 2H, J =8.9 Hz, H-2, H-6 Ph), 7.46 (t, 1H, J =7.7 Hz, H-7), 7.53 (d, 1H, J =8.3 Hz, H-8), 7.69 (t, 1H, J =7.9 Hz, H-6), 7.86 (d, 2H, J =8.9 Hz, H-3, H-5 Ph), 7.91 (s, 1H, H-2), 8.11 (d, 1H, J =7.5 Hz, H-5), 11.81 (s, 1H, 3-NH); LC-MS, m/z =323 [M+1]; Anal. calcd. for $C_{17}H_{14}N_4O_3$: C, 63.35; H, 4.38; N, 17.38. Found: C, 63.34; H, 4.36; N, 17.35.

[Quinazolin-4(3H)-ylidenehydrazone](1,3,5-trimethyl-1H-pyrazol-4-yl)acetic acid (2h**)**. Yield – 80.1%. M.p. – 168-170°C; 1 H-NMR, δ : 2.50 (s, 6H, 3,5-(CH_3)₂, 3.72 (s, 3H, N- CH_3), 7.75-7.35 (m, 3H, H-6, H-7, H-8), 8.08/7.99 (d, 1H, J =7.5 Hz, H-5), 8.47/8.28 (s, 1H, H-2); LC-MS, m/z =325 [M+1]; Anal. calcd. for $C_{16}H_{16}N_6O$: C, 59.25; H, 4.97; N, 25.91. Found: C, 59.24; H, 4.96; N, 25.89.

[Quinazolin-4(3H)-ylidenehydrazone](2-thienyl)acetic acid (2i**)**. Yield – 80.5%. M.p. – 166-168°C; 1 H-NMR, δ : 7.18 (t, 1H, J =4.4 Hz, H-4 Th.), 7.60-7.48 (m, 2H, H-6 Quin, H-5 Th.), 7.65 (d, 1H, J =2.5 Hz, H-8), 7.75 (d, 1H, H-3 Th.), 7.70 (t, 1H, J =7.1 Hz, H-6), 8.47/7.91 (d, 1H, J =8.9 Hz, H-5), 8.71/8.20 (s, 1H, H-2); LC-MS, m/z =299 [M+1]; Anal. calcd. for $C_{14}H_{10}N_4O_2S$: C, 56.37; H, 3.38; N, 18.78; S, 10.75. Found: C, 56.34; H, 3.36; N, 18.75; S, 10.74.

4-Phenyl-4-[quinazolin-4(3H)-ylidenehydrazone]butanoic acid (2j**)**. Yield – 62.5%. M.p. – 196-198°C; LC-MS,

m/z =321 [M+1]; Anal. calcd. for $C_{18}H_{16}N_4O_2$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.51; H, 5.06; N, 17.52.

4-(4-Methylphenyl)-4-[quinazolin-4(3H)-ylidenehydrazone]butanoic acid (2k**)**. Yield – 77.6%. M.p. – 208-210°C; 1 H-NMR, δ : 2.47 (t, 2H, J =8.3 Hz, CH_2CH_2-), 2.35 (s, 3H, CH_3), 3.29 (t, 2H, J =7.6 Hz, CH_2-CH_2-), 7.26 (d, 2H, J =8.3 Hz, H-3, H-5 Ph), 7.45 (t, 1H, J =8.1 Hz, H-7), 7.52 (d, 1H, J =7.8 Hz, H-8), 7.66 (t, 1H, J =7.3 Hz, H-6), 7.98 (m, 3H, H-2 Quin, H-2, H-6 Ph), 8.24 (d, 1H, J =7.6 Hz, H-5), 11.96 (s, 1H, 3-NH), 12.06 (s, 1H, $COOH$); LC-MS, m/z =335 [M+1]; Anal. calcd. for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.43; N, 16.76. Found: C, 68.27; H, 5.46; N, 16.78.

4-(4-Methoxyphenyl)-4-[quinazolin-4(3H)-ylidenehydrazone]butanoic acid (2l**)**. Yield – 77.7%. M.p. – 228-230°C; LC-MS, m/z =352 [M+1]; Anal. calcd. for $C_{19}H_{18}N_4O_3$: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.10; H, 5.16; N, 15.93.

4-Oxo-4-(methylphenyl)-2-[quinazolin-4(3H)-ylidenehydrazone]butanoic acid (2m**)**. Yield – 91.9%. M.p. – 222-224°C; LC-MS, m/z =349 [M+1]; Anal. calcd. for $C_{19}H_{16}NO_3$: C, 65.51; H, 4.63; N, 16.08. Found: C, 65.52; H, 4.64; N, 16.07.

The general method of synthesis of methyl (ethyl)[(quinazolin-4(3H)-ylidenehydrazone]carboxilate (**3a-3q**).

Add ethyloxocarboxilate or methyl 2,4-dioxocarb oxilate (0.005 Mol) to the suspension of 0.8 g (0.005 Mol) of (3H-quinazolin-4-ylidene)hydrazine (**1a**) in 15 ml of propan-2-ol, boil the resulting mixture for 45-60 min (in case of methyl 2,4-dioxocarboxilate keep at room temperature with stirring for 45-60 min). Cool the mixture dilute with water. Filter the precipitate formed and dry.

Ethyl 2-(quinazolin-4(3H)-ylidenehydrazone)propanoate (3a**)**. Yield – 49.3%. M.p. – 128-130°C; 1 H-NMR, δ : 1.38 (t, 3H, J =6.8 Hz, OCH_2CH_3), 2.31 (s, 3H, CH_3), 4.9 (q, 2H, J =7.0 Hz, OCH_2CH_3), 7.36 (t, 1H, J =7.4 Hz, H-7), 7.50 (d, 1H, J =7.6 Hz, H-8), 7.60 (t, 1H, J =8.4 Hz, H-6), 7.86 (s, 1H, H-2), 8.32 (d, 1H, J =7.0 Hz, H-5), 11.43 (s, 1H, NH); LC-MS, m/z =259 [M+1]; Anal. calcd. for $C_{13}H_{14}N_4O_2$: C, 60.46; H, 5.46; N, 21.69. Found: C, 60.43; H, 5.44; N, 21.65.

Ethyl 3-phenyl-2-[quinazolin-4(3H)-ylidenehydrazone]propanoate (3b**)**. Yield – 64.3%. M.p. – 118-120°C; 1 H-NMR, δ : 1.25 (t, 3H, J =6.8 Hz, OCH_2CH_3), 4.23 (m, 4H, - CH_2Ph , - OCH_2CH_3), 7.51 (m, 5H, H-2, H-3, H-4, H-5, H-6 Ph), 7.50 (t, 1H, J =7.0 Hz, H-7), 7.57 (d, 1H, J =7.4 Hz, H-8), 7.73 (t, 1H, J =6.8 Hz, H-6), 7.94 (s, 1H, H-2), 8.35 (d, 1H, J =7.4 Hz, H-5), 11.53 (s, 1H, NH); LC-MS, m/z =335 [M+1]; Anal. calcd. for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.43; N, 16.76. Found: C, 68.26; H, 5.46; N, 16.80.

Ethyl 3-(4-nitrophenyl)-2-[quinazolin-4(3H)-ylidenehydrazone]propanoate (3c**)**. Yield – 55.2%. M.p. – 108-110°C; 1 H-NMR, δ : 1.26 (t, 3H, J =7.4 Hz, OCH_2CH_3), 4.24 (q, 2H, J =6.0 Hz, OCH_2CH_3), 4.37 (s, 2H, CH_2Ph),

7.53 (m, 3H, H-7 Quin, H-2, H-4 Ph), 7.60 (d, 1H, $J=7.8$ Hz, H-8), 7.76 (t, 1H, $J=6.4$ Hz, H-6), 7.97 (s, 1H, H-2), 8.33 (d, 2H, $J=8.0$ Hz, H-3, H-5 Ph), 8.33 (d, 1H, $J=7.6$ Hz, H-5), 11.69 (s, 1H, NH); LC-MS, $m/z=380$ [M+1]; Anal. calcd. for $C_{19}H_{17}N_5O_4$: C, 60.15; H, 4.52; N, 18.46. Found: C, 60.12; H, 4.51; N, 18.43.

Ethyl phenyl-[quinazolin-4(3H)-ylidenehydrazone]acetate (3d). Yield – 78.8%. M.p. – 182–184°C; 1 H-NMR, δ : 1.45 (s, 3H, OCH_2CH_3), 4.29 (q, 2H, OCH_2CH_3), 7.52–7.44 (m, 5H, H-7, H-8 Quin, H-3, H-5 Ph), 7.71 (t, 1H, H-6), 7.92–7.85 (m, 2H, H-2, H-6 Ph), 7.94 (s, 1H, H-2), 8.12 (d, 1H, H-5), 11.86 (s, 1H, NH); LC-MS, $m/z=321$ [M+1]; Anal. calcd. for $C_{18}H_{16}N_4O_2$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.47; H, 5.01; N, 17.47.

Ethyl (4-methylphenyl)-[quinazolin-4(3H)-ylidenehydrazone]acetate (3e). Yield – 86.1%. M.p. – 146–148°C; 1 H-NMR, δ : 1.44 (t, 3H, $J=7.2$ Hz, OCH_2CH_3), 3.42 (s, 3H, CH_3), 4.45 (q, 2H, $J=6.9$ Hz, OCH_2CH_3), 7.22 (d, 2H, $J=7.7$ Hz, H-3, H-5 Ph), 7.33 (t, 1H, $J=7.9$ Hz Hz, H-7), 7.47 (d, 1H, $J=8.5$ Hz, H-8), 7.57 (t, 1H, $J=7.2$ Hz, H-6), 7.72 (d, 2H, $J=7.2$ Hz, H-2, H-6 Ph), 7.89 (s, 1H, H-2), 8.12 (d, 1H, $J=8.4$ Hz, H-5), 11.80 (s, 1H, NH); LC-MS, $m/z=335$ [M+1]; Anal. calcd. for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.43; N, 16.76. Found: C, 68.27; H, 5.47; N, 16.74.

Ethyl (4-methoxyphenyl)-[quinazolin-4(3H)-ylidenehydrazone]acetate (3f). Yield – 65.1%. M.p. – 196–198°C; 1 H-NMR, δ : 1.35 (t, 3H, $J=7.1$ Hz, OCH_2CH_3), 3.83 (s, 3H, OCH_3), 4.42 (q, 2H, $J=7.1$ Hz, OCH_2CH_3), 7.04 (d, 2H, $J=8.4$ Hz, H-3, H-5 Ph), 7.43 (t, 1H, $J=6.8$ Hz, H-7), 7.51 (d, 1H, $J=8.1$ Hz, H-8), 7.67 (t, 1H, $J=7.6$ Hz, H-6), 7.81 (d, 2H, $J=8.8$ Hz, H-2, H-6 Ph), 7.82 (s, 1H, H-2), 8.06 (d, 1H, $J=7.8$ Hz, H-5), 11.58 (s, 1H, NH); LC-MS, $m/z=351$ [M+1]; Anal. calcd. for $C_{19}H_{18}N_4O_3$: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.12; H, 5.17; N, 15.94.

Ethyl [quinazolin-4(3H)-ylidenehydrazone](2-thienyl)acetate (3g). Yield – 75.5%. M.p. – 100–102°C; 1 H-NMR, δ : 1.37 (t, 3H, $J=7.1$ Hz, OCH_2CH_3), 4.41 (q, 2H, $J=7.2$ Hz, OCH_2CH_3), 7.19 (t, 1H, $J=4.0$ Hz, H-7), 7.59 (m, 3H, H-8 Quin., H-4, H-5 Th.), 7.77 (t, 1H, $J=7.2$ Hz, H-6), 7.94 (m, 2H, H-2 Quin, H-3 Th.), 8.49 (d, 1H, $J=7.1$ Hz, H-5), 11.88 (s, 1H, NH); LC-MS, $m/z=327$ [M+1]; Anal. calcd. for $C_{16}H_{14}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17; S, 9.82. Found: C, 58.85; H, 4.29; N, 17.14; S, 9.79.

Ethyl 3-[quinazolin-4(3H)-ylidenehydrazone]butanoate (3h). Yield – 91.8%. M.p. – 168–170°C; 1 H-NMR, δ : 1.17 (t, 3H, CH_2-CH_3), 2.12 (s, 3H, CH_3), 4.12/3.99 (q, 2H, CH_2-CH_3), 4.42/4.12 (s, 1H, CH/CH_2), 7.61/7.24 (t, 1H, H-7), 7.38/7.12 (d, 1H, H-8), 7.72/7.43 (t, 1H, H-6), 8.18/7.88 (s, 1H, H-2), 8.06/7.81 (d, 1H, H-5), 11.46/11.22 (s, 1H, 3-NH), 13.84 (s, 1H, =NNH); LC-MS, $m/z=272$ [M+1]; Anal. calcd. for $C_{14}H_{16}N_4O_2$: C, 61.75; H, 5.92; N, 20.57. Found: C, 61.78; H, 5.96; N, 20.59.

Ethyl 3-phenyl-3-[quinazolin-4(3H)-ylidenehydrazone]propanoate (3i). Yield – 83.3%. M.p. – 192–194°C; 1 H-NMR, δ : 1.26 (t, 3H, CH_2-CH_3), 4.28 (q, 2H, CH_2-CH_3),

4.38 (s, 1H, =CH), 7.50–7.30 (m, 5H, H-5, H-6 Quin, H-3, H-4, H-5 Ph), 7.58 (t, 1H, H-6); 7.98 (s, 2H, H-2, H-6 Ph), 7.82 (s, 1H, H-2), 8.21 (d, 1H, H-5), 11.43 (s, 1H, 3-NH), 12.13 (s, 1H, =NNH); LC-MS, $m/z=335$ [M+1]; Anal. calcd. for $C_{19}H_{18}N_4O_2$: C, 68.25; H, 5.43; N, 16.76. Found: C, 68.27; H, 5.46; N, 16.79.

Ethyl 3-(2-furyl)-3-[quinazolin-4(3H)-ylidenehydrazone]propanoate (3j). Yield – 55.5%. M.p. – 158–160°C; 1 H-NMR, δ : 1.10 (t, 3H, $J=7.1$ Hz, OCH_2CH_3), 3.96 (s, 2H, CH_2), 4.03 (q, 2H, $J=7.1$ Hz, OCH_2CH_3), 6.67 (t, 1H, $J=1.7$ Hz, H-4 Fur), 7.21 (d, 1H, $J=3.2$ Hz, H-3 Fur), 7.42 (t, 1H, $J=8.0$ Hz, H-7), 7.49 (d, 1H, $J=8.1$ Hz, H-8), 7.65 (t, 1H, $J=8.1$ Hz, H-6), 7.84 (s, 1H, H-5 Fur), 7.88 (s, 1H, H-2), 8.13 (d, 1H, $J=8.1$ Hz, H-5), 11.54 (s, 1H, 3-NH); LC-MS, $m/z=325$ [M+1]; Anal. calcd. for $C_{17}H_{16}N_4O_3$: C, 62.95; H, 4.97; N, 17.27. Found: C, 62.93; H, 4.92; N, 17.24.

Ethyl 4-phenyl-4-[quinazolin-4(3H)-ylidenehydrazone]butanoate (3k). Yield – 52.3%; oil; LC-MS, $m/z=349$ [M+1]; Anal. calcd. for $C_{20}H_{20}N_4O_2$: C, 68.95; H, 5.79; N, 16.08. Found: C, 68.97; H, 5.81; N, 16.11.

Ethyl 4-(4-methylphenyl)-4-[quinazolin-4(3H)-ylidenehydrazone]butanoate (3l). Yield – 48.0%. M.p. – 166–168°C; 1 H-NMR, δ : 1.11 (t, 3H, $J=7.1$ Hz, OCH_2CH_3), 2.49 (t, 2H, $J=7.8$ Hz, CH_2CH_2CO), 2.54 (t, 2H, $J=7.8$ Hz, CH_2CH_2CO), 2.58 (s, 3H, CH_3), 4.00 (q, 2H, $J=7.1$ Hz, OCH_2CH_3), 6.98 (d, 2H, $J=8.8$ Hz, H-3, H-5 Ph), 7.41 (t, 1H, $J=7.6$ Hz, H-7), 7.47 (d, 1H, $J=7.6$ Hz, H-8), 7.63 (t, 1H, $J=7.8$ Hz, H-6), 7.83 (s, 1H, H-2), 8.00 (d, 2H, $J=7.3$ Hz, H-2, H-6 Ph), 8.20 (d, 1H, $J=7.8$ Hz, H-5), 11.40 (s, 1H, NH); Anal. calcd. for $C_{21}H_{22}N_4O_2$: C, 69.59; H, 6.12; N, 15.46. Found: C, 69.57; H, 6.10; N, 15.43.

Ethyl 4-(4-methoxyphenyl)-4-[quinazolin-4(3H)-ylidenehydrazone]butanoate (3m). Yield – 52.9%. M.p. – 162–164°C; 1 H-NMR, δ : 1.12 (t, 3H, $J=7.1$ Hz, OCH_2CH), 2.49 (t, 2H, $J=7.8$ Hz, CH_2CH_2-CO), 2.54 (t, 2H, $J=7.8$ Hz, CH_2CH_2CO), 3.53 (s, 3H, CH_3), 4.00 (q, 2H, $J=7.1$ Hz, OCH_2CH_3), 7.24 (d, 2H, $J=7.6$ Hz, H-3, H-5 Ph), 7.42 (t, 1H, $J=7.6$ Hz, H-7), 7.48 (d, 1H, $J=7.6$ Hz, H-8), 7.64 (t, 1H, $J=7.8$ Hz, H-6), 7.83 (s, 1H, H-2), 7.95 (d, 2H, $J=8.8$ Hz, H-2, H-6 Ph), 8.20 (d, 1H, $J=7.8$ Hz, H-5), 11.40 (s, 1H, NH); LC-MS, $m/z=379$ [M+1]; Anal. calcd. for $C_{21}H_{22}N_4O_3$: C, 66.65; H, 5.86; N, 14.80. Found: C, 66.61; H, 5.83; N, 14.78.

Methyl 4-oxo-4-phenyl-2-[quinazolin-4(3H)-ylidenehydrazone]butanoate (3n). Yield – 65.9%. M.p. – 120–122°C; 1 H-NMR, δ : 3.91/3.81 (s, 3H, -OCH₃), 4.59 (s, 2H, -CH₂), 5.98 (s, 1H, =CH-), 7.31/7.27 (t, 1H, $J=7.6$ Hz, H-7), 7.45/7.23 (d, 1H, $J=8.3$ Hz, H-8), 7.56–7.49 (m, 3H, H-3, H-4, H-5 Ph), 7.67 (t, 1H, $J=7.3$ Hz, H-6), 7.93/7.85 (d, 2H, $J=8.1$ Hz H-2, H-6 Ph), 8.03 (d, 1H, $J=8.1$ Hz, H-5), 8.11 (s, 1H, H-2), 11.94/11.74 (s, 1H, 3-NH), 13.72 (s, 1H, HO-C=); LC-MS, $m/z=349$ [M+1]; Anal. calcd. for $C_{19}H_{16}N_4O_3$: C, 65.51; H, 4.63; N, 13.78. Found: C, 65.55; H, 4.65; N, 13.81.

Methyl 4-oxo-4-(4-methylphenyl)-2-[quinazolin-4(3H)-ylidenehydrazone]butanoate (3o). Yield – 58.1%.

M.p. – 110–112°C; $^1\text{H-NMR}$, δ : 2.39/2.34 (s, 3H, -CH₃), 3.91/3.81 (s, 3H, -OCH₃), 4.56 (s, 2H, -CH₂-), 5.95 (s, 1H, =CH-), 7.93–7.20 (m, 8H, H-5, H-6, H-7, H-8 Quin, H-2, H-6, H-3, H-5 Ph), 8.09 (s, 1H, H-2), 11.91/11.70 (s, 1H, 3-NH), 13.66 (s, 1H, HO-C=); LC-MS, m/z =363 [M+1]; Anal. calcd. for C₂₀H₁₈N₄O₃: C, 66.26; H, 5.01; N, 15.46. Found: C, 66.23; H, 4.98; N, 15.42.

Methyl 4-oxo-4-(4-methoxyphenyl)-2-[quinazolin-4(3H)-ylidenehydrazone]butanoate (3p). Yield – 61.0%. M.p. – 152–153°C; $^1\text{H-NMR}$, δ : 3.81/3.80 (s, 3H, Ph-OCH₃), 3.90/3.85 (s, 3H, -OCH₃), 4.55 (s, 2H, -CH₂-), 7.07 (d, 2H, J =8.8 Hz, H-3, H-5 Ph), 5.93 (s, 1H, =CH-), 7.21/6.99 (d, 1H, J =8.8 Hz, H-8), 7.30 (t, 1H, J =6.6 Hz, H-7), 7.51 (t, 1H, J =8.6 Hz, H-6), 7.93/7.73 (d, 1H, J =7.8 Hz, H-5), 8.00/7.90 (d, 2H, J =8.8 Hz, H-2, H-6, Ph), 8.07 (s, 1H, H-2), 11.67 (s, 1H, 3-NH), 13.60 (s, 1H, HO-C=); LC-MS, m/z =379 [M+1]; Anal. calcd. for C₂₀H₁₈N₄O₄: C, 63.49; H, 4.79; N, 14.81. Found: C, 64.51; H, 4.81; N, 14.82.

Methyl 4-oxo-2-[quinazolin-4(3H)-ylidenehydrazone]-4-(2-thienyl)butanoate (3q). Yield – 48.6%. M.p. – 181–183°C; $^1\text{H-NMR}$, δ : 3.91/3.82 (s, 3H, -OCH₃), 4.55 (s, 2H, -CH₂-), 5.88 (s, 1H, =CH-), 7.30/7.15 (t, 1H, J =5.1 Hz, H-4 Th.), 7.30/7.22 (d, 1H, J =7.6 Hz, H-6), 7.68/7.52 (t, 1H, J =7.1 Hz, H-7), 7.79 (d, 1H, J =4.6 Hz, H-5 Th.), 8.03/7.84 (d, 1H, J =8.1 Hz, H-5), 7.94 (s, 1H, H-2), 8.12/8.07 (d, 1H, J =3.7 Hz, H-3 Th.), 11.91/11.74 (s, 1H, 3-NH), 13.44 (s, 1H, HO-C=); LC-MS, m/z =355 [M+1]; Anal. calcd. for C₁₇H₁₄N₄O₃S: C, 57.62; H, 3.98; N, 15.81; S, 9.05. Found: C, 57.59; H, 3.96; N, 15.79; S, 9.02.

The general method of synthesis of N-izopropylidene-N'-(quinazolin-4(3H)-ylidene)-hydrazine (4a).

Method A. Add 3-oxocarboxylic acid (0.01 Mol) to the solution of 1.6 g (0.01 Mol) of 4-hydrazino-quinazoline (**2c**) in 15 ml of propan-2-ol and allow to stand at room temperature. During the reaction carbon monoxide is released. At the end of the process (2 h) filter the precipitate and dried.

Method B. To 2.3 g (0.014 Mol) of 4-hydrazino-quinazoline (**2c**) add 20 ml of acetone and 2 drops of the concentrated hydrochloric acid and boil for 90 min. Cool the solution, filter the precipitate and dry.

N-izopropylidene-N'-(quinazolin-4(3H)-ylidene)hydrazine (4a). Yield – 50.4%. M.p. – 166–168°C; $^1\text{H-NMR}$, δ : 2.09 (s, 3H, CH₃), 2.14 (s, 3H, CH₃), 7.24 (t, 1H, J =7.6 Hz, H-7), 7.38 (d, 1H, J =8.1 Hz, H-8), 7.47 (t, 1H, J =7.1 Hz, H-6), 8.17 (d, 1H, J =8.1 Hz, H-5), 7.65 (s, 1H, H-2), 10.7 (s, 1H, 3-NH); LC-MS, m/z =201 [M+1]; Anal. calcd. for C₁₁H₁₂N₄: C, 65.98; H, 6.04; N, 27.98. Found: C, 65.96; H, 6.02; N, 27.96.

The general method of synthesis of [(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)-hydrazono] carboxylic acids (5.1–5.9).

To the suspension of 0.88 g (0.005 Mol) of 4-hydrazino-1*H*-quinazolin-2-one (**1b**) in 10 ml of methanol add 0.005 Mol of the appropriate α -oxocarboxy-

lic acid and 1.03 g (0.005 Mol) of 4-(4'-methylphenyl)-2,4-dioxobutanoic acid, 1-2 drops of the concentrated hydrochloric acid and boil for 15 min, cool, and filter the precipitate.

2-[(2-Oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazono]propanoic acid (5a). Yield – 77.2%. M.p. – 252–254°C; $^1\text{H-NMR}$, δ : 2.26 (s, 3H, CH₃), 7.16 (m, 2H, H-6, H-8), 7.55 (t, 1H, J =7.6 Hz, H-7), 8.18 (d, 1H, J =8.2 Hz, H-5), 11.05 (s, 1H, 3-NH), 11.40 (s, 1H, 1-NH), 12.37 (s, 1H, COOH); LC-MS, m/z =247 [M+1]; Anal. calcd. for C₁₁H₁₀N₄O₃: C, 53.66; H, 4.09; N, 22.75. Found: C, 55.63; H, 4.03; N, 22.73.

2-[2-Oxo-2,3-dihydroquinazolin-4(1H)-ylidene]hydrazono]pentanedioic acid (5b). Yield – 72.4%. M.p. – 242–244°C; $^1\text{H-NMR}$, δ : 2.98 (t, 2H, J =7.8 Hz, -CH₂-CH₂-COOH), 3.33 (t, 2H, -CH₂-COOH), 7.13 (d, 1H, J =8.1 Hz, H-8), 7.53 (t, 1H, J =7.8 Hz, H-7), 7.58 (t, 1H, J =6.8 Hz, H-6), 8.10 (d, 1H, J =8.1 Hz, H-5), 11.03 (s, 1H, 3-NH), 11.36 (s, 1H, 1-NH), 12.28 (s, 1H, COOH); LC-MS, m/z =305 [M+1]; Anal. calcd. for C₁₃H₁₂N₄O₅: C, 51.32; H, 3.98; N, 18.41. Found: C, 51.36; H, 4.01; N, 18.43.

3-(4-Nitrophenyl)-2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazono]propanoic acid (5c). Yield – 81.7%. M.p. – 250–252°C; LC-MS, m/z =368 [M+1]; Anal. calcd. for C₁₇H₁₃N₅O₅: C, 55.59; H, 3.57; N, 19.07. Found: C, 55.54; H, 3.53; N, 19.03.

Phenyl-[2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene]hydrazono]acetic acid (5d). Yield – 77.6%. M.p. – 280–282°C; $^1\text{H-NMR}$, δ : 7.13 (m, 2H, H-7, H-8), 7.51 (m, 5H, H-6 Quin, H-2, H-3, H-5, H-6 Ph), 8.04 (m, 2H, H-5 Quin, H-4 Ph), 11.06/10.62 (s, 1H, 3-NH), 11.56/10.85 (s, 1H, 1-NH), 12.63 (s, 1H, COOH); LC-MS, m/z =309 (80:20) [M+1]; Anal. calcd. for C₁₆H₁₂N₄O₃: C, 62.31; H, 3.92; N, 18.17. Found: C, 62.35; H, 3.93; N, 18.21.

(4-Methylphenyl)-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazono]acetic acid (5e). Yield – 87.0%. M.p. – 266–268°C; $^1\text{H-NMR}$, δ : 2.35 (s, 3H, CH₃), 7.10 (m, 2H, H-7, H-8), 7.44 (d, 2H, J =8.0 Hz, H-3, H-5 Ph), 7.53 (t, 1H, J =7.0 Hz, H-6), 7.91 (d, 2H, H-3, J =8.4 Hz, H-5 Ph), 7.98 (d, 1H, J =7.6 Hz, H-5), 11.09/10.59 (s, 1H, 3-NH), 11.60/10.82 (s, 1H, 1-NH), 12.66 (s, 1H, COOH); LC-MS, m/z =323 (50:50) [M+1]; Anal. calcd. for C₁₇H₁₄N₄O₃: C, 63.35; H, 4.38; N, 17.38. Found: C, 63.35; H, 4.39; N, 17.38.

(4-Methoxyphenyl)-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazono]acetic acid (5f). Yield – 82.8%. M.p. – 280–282°C; $^1\text{H-NMR}$, δ : 3.84 (s, 3H, OCH₃), 7.03 (d, 2H, H-3, H-5 Ph), 7.15 (m, 2H, H-7, H-8), 7.53 (t, 1H, J =7.5 Hz, H-6), 7.98 (m, 2H, H-5 Quin, H-3, H-5 Ph), 11.03/10.47 (s, 1H, 3-NH), 11.42/10.77 (s, 1H, 1-NH), 11.49 (s, 1H, COOH); LC-MS, m/z =339 (50:50) [M+1]; Anal. calcd. for C₁₇H₁₄N₄O₄: C, 60.35; H, 4.17; N, 16.56. Found: C, 60.39; H, 4.21; N, 16.58.

*[2-Oxo-2,3-dihydroquinazolin-4(1H)-ylidene]hydrazono]-[1,3,5-trimethyl-1*H*-pyrazol-4-yl]acetic acid (5g).* Yield – 74.7%. M.p. – 252–254°C; $^1\text{H-NMR}$, δ : 2.30 (s,

3H, 5-CH₃), 2.42 (s, 3H, 3-CH₃), 3.73 (s, 3H, N-CH₃), 7.14 (m, 2H, H-6, H-8), 7.53 (t, 1H, J=6.8 Hz, H-7), 7.92/7.86 (d, 1H, J=8.1 Hz, H-5), 9.22 (s, 1H, 3-NH), 10.92 (s, 1H, 1-NH), 12.55 (s, 1H, COOH); LC-MS, m/z =341 (50:50) [M+1]; Anal. calcd. for C₁₆H₁₆N₆O₃: C, 56.47; H, 4.74; N, 24.69. Found: C, 56.45; H, 4.73; N, 24.65.

*[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone](2-thienyl)acetic acid (**5h**).* Yield – 85.9%. M.p. – 206–208°C; ¹H-NMR, δ: 7.31–7.11 (m, 3H, H-7, H-8 Quin, H-4 Th.), 7.65/7.56 (t, 1H, J=7.3 Hz, H-6), 7.80/7.49 (t, 1H, H-5), 7.93 (d, 1H, J=5.2 Hz, H-3 Th.), 8.38 (d, 1H, J=4.4 Hz, H-5 Th.), 10.94 (s, 1H, 3-NH), 11.20 (s, 1H, 1-NH), 11.63 (s, 1H, COOH); LC-MS, m/z =314 (50:50) [M+1]; Anal. calcd. for C₁₄H₁₀N₄O₃S: C, 53.50; H, 3.21; N, 17.82; S, 10.20. Found: C, 53.45; H, 3.17; N, 17.79; S, 10.17.

*4-(4-Methylphenyl)-4-oxo-2-[(2Z)-(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]butanoic acid (**5i**).* Yield – 73.1%. M.p. – 224–228°C; ¹H-NMR, δ: 4.50 (s, 2H, CH₂), 6.97 (t, 1H, J=7.6 Hz, H-7), 7.09 (d, 1H, J=8.3 Hz, H-8), 7.33 (d, 2H, J=8.1 Hz, H-3, H-5 Ph), 7.52 (t, 1H, J=7.1 Hz, H-6), 7.72 (d, 1H, J=7.3 Hz, H-5), 7.89 (d, 2H, J=8.1 Hz, H-2, H-6 Ph), 11.07 (s, 1H, 3-NH), 11.45 (s, 1H, 1-NH), 12.55 (s, 1H, COOH); LC-MS, m/z =363 [M+1]; Anal. calcd. for C₁₉H₁₆N₄O₄: C, 62.63; H, 4.43; N, 15.38. Found: C, 62.59; H, 3.17; N, 17.79; S, 10.17.

The general method of synthesis of methyl (ethyl) 2-R-2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]carboxylic acids (6a**–**6p**).**

To the suspension of 0.88 g (0.005 Mol) of 4-hydrazino-1*H*-quinazolin-2-one (**1.2**) in 15 ml of propan-2-ol add 0.005 Mol of the corresponding oxo-carboxylic ester or 2,4-dioxo-4-aryl(hetaryl)butanoic acid, 1-2 drops of the concentrated hydrochloric acid and boil for 1 h. Cool the suspension, filter the precipitate formed and dry.

*Ethyl 2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]propanoate (**6a**).* Yield – 65.7%. M.p. – 248–250°C; LC-MS, m/z=275 [M+1]; Anal. calcd. for C₁₃H₁₄N₄O₃: C, 56.93; H, 5.14; N, 20.43. Found: C, 56.90; H, 5.11; N, 20.39.

*Ethyl 2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]-3-phenylpropanoate (**6b**).* Yield – 57.1%. M.p. – 192–194°C; LC-MS, m/z=351 [M+1]; Anal. calcd. for C₁₉H₁₈N₄O₃: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.10; H, 5.15; N, 15.96.

*Ethyl 3-(4-nitrophenyl)-2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]propanoate (**6c**).* Yield – 79.9%. M.p. – 242–244°C; LC-MS, m/z=396 [M+1]; Anal. calcd. for C₁₉H₁₇N₅O₅: C, 57.72; H, 4.33; N, 17.71. Found: C, 57.76; H, 4.38; N, 17.79.

*Ethyl [(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone](phenyl)acetate (**6d**).* Yield – 62.3%. M.p. – 266–268°C; ¹H-NMR, δ: 1.36 (t, 3H, J=7.0 Hz, -OCH₂CH₃), 4.44 (q, 2H, J=7.0 Hz, OCH₂CH₃), 7.12 (m, 2H, H-7, H-8 Quin), 7.51 (m, 4H, H-6 Quin, H-3, H-4,

H-5 Ph), 7.95 (d, 1H, J=7.8 Hz, H-5), 8.00 (d, 2H, J=6.4 Hz, H-3, H-5 Ph), 10.72 (s, 1H, 3-NH), 10.89 (s, 1H, 1-NH); LC-MS, m/z=337 [M+1]; Anal. calcd. for C₁₈H₁₆N₄O₃: C, 64.28; H, 4.79; N, 16.66. Found: C, 64.26; H, 4.81; N, 16.69.

*Ethyl (4-methylphenyl)[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]acetate (**6e**).* Yield – 40.0%. M.p. – 257–259°C; ¹H-NMR, δ: 1.33 (t, 3H, J=7.0 Hz, -OCH₂CH₃), 3.31 (s, 3H, CH₃) 4.43 (q, 2H, J=7.0 Hz, OCH₂CH₃), 7.09 (m, 2H, H-7, H-8 Quin), 7.29 (d, 2H, J=7.8 Hz, H-2, H-6 Ph), 7.52 (t, 1H, J=7.6 Hz, H-7), 7.89 (m, 3H, H-5 Quin, H-3, H-5 Ph), 10.65 (s, 1H, 3-NH), 10.84 (s, 1H, 1-NH); LC-MS, m/z=351 [M+1]; Anal. calcd. for C₁₉H₁₈N₄O₃: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.16; H, 5.20; N, 16.05.

*Ethyl (4-methoxyphenyl)[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]acetate (**6f**).* Yield – 71.0%. M.p. – 264–266°C; ¹H-NMR, δ: 1.43 (t, 3H, J=7.0 Hz, OCH₂CH₃), 3.85 (s, 3H, OCH₃), 4.44 (q, 2H, J=7.2 Hz, OCH₂CH₃), 6.97 (d, 2H, J=8.7 Hz, H-3, H-5 Ph), 7.03 (t, 1H, J=8.2 Hz, H-7), 7.10 (d, 1H, J=8.0 Hz, H-8), 7.40 (t, 1H, J=8.0 Hz, H-6), 7.80 (d, 2H, J=8.6 Hz, H-2, H-6 Ph), 7.96 (d, 1H, J=7.4 Hz, H-5), 9.83 (s, 1H, 3-NH), 10.76 (s, 1H, 1-NH); LC-MS, m/z=367 [M+1]; Anal. calcd. for C₁₉H₁₈N₄O₄: C, 62.29; H, 4.95; N, 15.29. Found: C, 62.32; H, 4.98; N, 15.32.

*Ethyl [(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone](2-thienyl)acetate (**6g**).* Yield – 52.6%. M.p. – 218–220°C; ¹H-NMR, δ: 1.36 (t, 3H, J=7.0 Hz, OCH₂CH₃), 4.40 (q, 2H, J=7.2 Hz, OCH₂CH₃), 7.26–7.14 (m, 3H, H-7, H-8 Quin, H-4 Th.), 7.60 (t, 1H, J=7.0 Hz, H-6 Quin), 7.70 (d, 1H, J=3.1 Hz, H-5 Th.), 7.95 (d, 1H, J=4.9 Hz, H-3 Th.), 8.30 (d, 1H, J=7.6 Hz, H-5), 10.00 (s, 1H, 3-NH), 11.07 (s, 1H, 1-NH); LC-MS, m/z=343 [M+1]; EI-MS, m/z (I_{rel}, %) = 344 (3.5), 343 (12.7), 342 (53.8), 271 (5.9), 270 (14.9), 269 (100.0), 145 (6.7), 144 (5.6), 132 (9.1), 125 (13.9), 118 (6.8), 117 (5.4), 110 (6.4), 105 (8.6), 90 (15.3); Anal. calcd. for C₁₆H₁₄N₄O₃: C, 51.13; H, 4.12; N, 16.36; S, 9.37. Found: C, 51.16; H, 4.15; N, 16.39; S, 9.40.

*Ethyl 3-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]butanoate (**6h**).* Yield – 48.6%. M.p. – 192–194°C; ¹H-NMR, δ: 1.22/1.14 (t, 3H, J=7.1 Hz, OCH₂CH₃), 2.10 (s, 3H, CH₃), 3.60/3.49 (s, 2H, CH/CH₂), 4.13/4.03 (q, 2H, J=7.1 Hz, OCH₂CH₃), 7.07 (m, 2H, H-7, H-8), 7.49 (t, 1H, J=7.6 Hz, H-6), 8.00/7.95 (d, 1H, J=7.6 Hz, H-5), 9.39 (s, 1H, 3-NH), 9.49 (s, 1H, =NNH), 10.71 (s, 1H, 1-NH); LC-MS, m/z=289 [M+1]; Anal. calcd. for C₁₄H₁₆N₄O₃: C, 58.33; H, 5.59; N, 19.43. Found: C, 58.36; H, 5.61; N, 19.46.

*Ethyl 3-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]-3-phenylpropanoate (**6i**).* Yield – 57.0%. M.p. – 216–218°C; LC-MS, m/z=351 [M+1]; Anal. calcd. for C₁₉H₁₈N₄O₃: C, 65.13; H, 5.18; N, 15.99. Found: C, 65.16; H, 5.21; N, 16.02.

*Ethyl 3-(2-furyl)-3-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]propanoate (**6j**).* Yield – 52.9%.

M.p. – 230–232°C; $^1\text{H-NMR}$, δ : 1.11 (t, 3H, $J=7.1$ Hz, OCH_2CH_3), 3.94 (s, 2H, CH_2), 4.04 (q, 2H, $J=7.1$ Hz, OCH_2CH_3), 6.65 (t, 1H, $J=1.5$ Hz, H-4 Fur), 7.08 (d, 1H, $J=7.6$ Hz, H-8), 7.13 (t, 1H, $J=7.6$ Hz, H-7), 7.42 (d, 1H, $J=3.2$ Hz, H-3 Fur), 7.50 (t, 1H, $J=7.6$ Hz, H-6), 7.85 (s, 1H, H-5 Fur), 8.00 (d, 1H, $J=7.6$ Hz, H-5), 9.77 (s, 1H, 3-NH), 10.84 (s, 1H, 1-NH); LC-MS, m/z =341 [M+1]; Anal. calcd. for $\text{C}_{17}\text{H}_{16}\text{N}_4\text{O}_3$: C, 60.00; H, 4.74; N, 16.46. Found: C, 60.06; H, 4.76; N, 16.51.

Methyl 4-oxo-2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]-4-phenylbutanoate (6k). Yield – 65.9%. M.p. – 228–230°C; $^1\text{H-NMR}$, δ : 3.80 (s, 3H, $-\text{OCH}_3$), 4.57 (s, 2H, $-\text{CH}_2-$), 6.97 (t, 1H, $J=7.6$ Hz, H-7), 7.08 (d, 1H, $J=8.3$ Hz, H-8), 7.67 (t, 1H, $J=7.1$ Hz, H-6), 7.56–7.50 (m, 3H, H-3, H-4, H-5 Ph), 7.71 (d, 1H, $J=8.1$ Hz, H-5), 8.01 (d, 2H, $J=8.1$ Hz, H-2, H-6 Ph), 9.62 (s, 1H, 3-NH), 11.07 (s, 1H, 1-NH); LC-MS, m/z =365 [M+1]; Anal. calcd. for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{O}_4$: C, 62.63; H, 4.43; N, 15.38. Found: C, 62.67; H, 4.47; N, 15.41.

Methyl 4-(4-methylphenyl)-4-oxo-2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]butanoate (6l). Yield – 58.1%. M.p. – 235–237°C; $^1\text{H-NMR}$, δ : 2.39 (s, 3H, $-\text{CH}_3$), 3.80 (s, 3H, $-\text{OCH}_3$), 4.53 (s, 2H, $-\text{CH}_2-$), 6.97 (t, 1H, $J=7.6$ Hz, H-7), 7.09 (d, 1H, $J=8.3$ Hz, H-8), 7.34 (d, 2H, $J=8.1$ Hz, H-3, H-5 Ph), 7.51 (t, 1H, $J=7.1$ Hz, H-6), 7.72 (d, 1H, $J=7.8$ Hz, H-5), 7.90 (d, 2H, $J=8.1$ Hz, H-2, H-6 Ph), 9.53 (s, 1H, 3-NH), 11.05 (s, 1H, 1-NH); LC-MS, m/z =379 [M+1]; Anal. calcd. for $\text{C}_{20}\text{H}_{18}\text{N}_4\text{O}_4$: C, 63.49; H, 4.79; N, 14.81. Found: C, 63.52; H, 4.83; N, 14.84.

Methyl 4-(4-methoxyphenyl)-4-oxo-2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]butanoate (6m). Yield – 61.0%. M.p. – 222–224°C; $^1\text{H-NMR}$, δ : 3.79 (s, 3H, $-\text{OCH}_3$), 3.85 (s, 3H, $-\text{OCH}_3$), 4.52 (s, 2H, $-\text{CH}_2-$), 6.99 (t, 1H, $J=7.6$ Hz, H-7), 7.05 (d, 2H, $J=8.6$ Hz, H-3, H-5 Ph), 7.07 (d, 1H, $J=8.6$ Hz, H-8), 7.52 (t, 1H, $J=7.3$ Hz, H-6), 7.77 (d, 1H, $J=7.8$ Hz, H-5), 8.00 (d, 2H, $J=8.8$ Hz, H-2, H-6 Ph), 9.60 (s, 1H, 3-NH), 11.06 (s, 1H, 1-NH); LC-MS, m/z =394 [M+1]; Anal. calcd. for $\text{C}_{20}\text{H}_{18}\text{N}_4\text{O}_5$: C, 60.91; H, 4.60; N, 14.21. Found: C, 60.89; H, 4.58; N, 14.18.

Methyl 4-(4-chlorophenyl)-4-oxo-2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]butanoate (6n). Yield – 45.1%. M.p. – 238–240°C; $^1\text{H-NMR}$, δ : 3.81 (s, 3H, $-\text{OCH}_3$), 4.55 (s, 2H, $-\text{CH}_2-$), 6.97 (t, 1H, $J=7.8$ Hz, $J'=1.2$ Hz, H-7), 7.06 (d, 2H, $J=8.6$ Hz, H-3, H-5 Ph), 7.07 (d, 1H, $J=8.3$ Hz, H-8), 7.52 (t, 1H, $J=7.3$ Hz, $J'=1.5$ Hz, H-6), 7.69 (d, 1H, $J=7.8$ Hz, H-5), 8.01 (d, 2H, $J=8.6$ Hz, H-2, H-6 Ph), 9.59 (s, 1H, 3-NH), 11.08 (s, 1H, 1-NH); LC-MS, m/z =399 [M+1]; Anal. calcd. for $\text{C}_{19}\text{H}_{15}\text{ClN}_4\text{O}_4$: C, 57.22; H, 3.79; Cl, 8.89; N, 14.05. Found: C, 57.26; H, 3.81; Cl, 8.92; N, 14.08.

Methyl 4-(2-furyl)-4-oxo-2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]butanoate (6o). Yield – 56.5%. M.p. – 224–226°C; $^1\text{H-NMR}$, δ : 3.81 (s, 3H, $-\text{OCH}_3$), 4.37 (s, 2H, $-\text{CH}_2-$), 6.76 (d, 1H, $J=1.7$ Hz, H-3 Fur), 7.01 (t, 1H, H-6, $J^2=0.7$ Hz, $J=7.3$ Hz), 7.09 (t, 1H,

$J=8.3$ Hz, H-7), 7.56 (d, 1H, $J=3.4$ Hz, H-5 Fur), 7.67 (dd, 1H, $J=8.1$ Hz, $J^2=1.0$ Hz, H-5), 7.94 (t, 1H, H-6, $J^2=1.5$ Hz, $J=7.3$ Hz), 8.02 (d, 1H, $J=1.0$ Hz, H-3 Fur), 9.65 (s, 1H, 3-NH), 11.07 (s, 1H, 1-NH); LC-MS, m/z =355 [M+1]; Anal. calcd. for $\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}_5$: C, 57.63; H, 3.98; N, 15.81. Found: C, 57.66; H, 4.02; N, 15.83.

Methyl 4-(2-thienyl)-4-oxo-2-[(2-oxo-2,3-dihydroquinazolin-4(1H)-ylidene)hydrazone]butanoate (6p). Yield – 48.6%. M.p. – 240–241°C; $^1\text{H-NMR}$, δ : 3.81 (s, 3H, $-\text{OCH}_3$), 4.52 (s, 2H, $-\text{CH}_2-$), 6.98 (d, 1H, $J=7.6$ Hz, H-7), 7.09 (d, 1H, $J=7.8$ Hz, H-8), 7.28 (t, 1H, $J=4.4$ Hz, $J^2=1.0$ Hz, H-4 Th.), 7.52 (t, 1H, $J=7.6$ Hz, $J^2=1.2$ Hz, H-6), 7.67 (d, 1H, $J=7.6$ Hz, H-5), 8.03 (d, 1H, $J=7.3$ Hz, $J^2=1.5$ Hz, H-5 Th.), 8.11 (d, 1H, H-3 Th), 9.63 (s, 1H, 3-NH), 11.07 (s, 1H, 1-NH); LC-MS, m/z =371 [M+1]; Anal. calcd. for $\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}_4$: C, 55.13; H, 3.81; N, 15.13; S, 8.66. Found: C, 55.16; H, 3.84; N, 15.19; S, 8/69.

The general method of synthesis of 3-R-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (7a–7g).

Method A. Heat esters 3a–3g (0.005 Mol) in 10 ml of glacial acetic acid for 6 h. Remove the solvent under the vacuum, add 5 ml of methanol to the residue, filter the precipitate, wash on the filter with diethyl ether and dry.

Method B. Add carbonyldiimidazole (0.0055 Mol) to the suspension of the corresponding 2-aryl-2-[(3H-quinazolin-4-ylidene)hydrazone]acetic acid (**2c–2f, 2i**) (0.005 Mol) in 10 ml of anhydrous dioxane or DMF. Heat the mixture at 80°C for 1 h, cool, filter the precipitate formed and dry.

3-Methyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (7a). Yield – Method A, 62.9%; method B, 82.6%. M.p. – 242–244°C; $^1\text{H-NMR}$, δ : 2.50 (s, 3H, CH_3), 7.79 (t, 1H, $J=7.6$ Hz, H-10), 7.90 (d, 1H, $J=8.0$ Hz, H-8), 8.02 (t, 1H, $J=7.8$ Hz, H-9), 8.53 (d, 1H, $J=7.8$ Hz, H-11), 8.94 (s, 1H, H-6); $^{13}\text{C-NMR}$, δ : 18.1; 120.2, 125.8, 128.2, 129.5, 135.8, 144.3, 144.5, 152.1, 156.5, 161.3. LC-MS, m/z =213 [M+1]; Anal. calcd. for $\text{C}_{11}\text{H}_8\text{N}_4\text{O}$: C, 62.26; H, 3.80; N, 26.40. Found: C, 62.25; H, 3.79; N, 26.37.

3-Benzyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (7b). Yield – Method A, 86.8%; method B, 89.3%. M.p. – 195–196°C; $^1\text{H-NMR}$, δ : 4.08 (s, 2H, CH_2), 7.37–7.24 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ph), 7.76 (t, 1H, $J=7.4$ Hz, H-10), 7.90 (d, 1H, $J=7.2$ Hz, H-8), 8.00 (t, 1H, $J=7.4$ Hz, H-9), 8.52 (d, 1H, $J=7.2$ Hz, H-11), 8.90 (s, 1H, H-6); LC-MS, m/z =289 [M+1]; Anal. calcd. for $\text{C}_{17}\text{H}_{12}\text{N}_4\text{O}$: C, 70.82; H, 4.20; N, 19.43. Found: C, 70.85; H, 4.23; N, 19.44.

3-(4-Nitrobenzyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (7c). Yield – Method A, 85.1%; method B, 84.7%. M.p. – 266–268°C; $^1\text{H-NMR}$, δ : 4.29 (s, 2H, CH_2), 7.66 (d, 2H, $J=7.8$ Hz, H-2, H-6 Ph), 7.78 (t, 1H, $J=7.2$ Hz, H-10), 7.91 (d, 1H, $J=7.6$ Hz, H-8), 8.01 (t, 1H, $J=8.4$ Hz, H-9), 8.15 (d, 2H, $J=8.4$ Hz, H-3, H-5 Ph), 8.55 (d, 1H, $J=8.4$ Hz, H-11), 8.80 (s, 1H, H-6); LC-MS, m/z =335 [M+1]; Anal. calcd. for $\text{C}_{17}\text{H}_{11}\text{N}_5\text{O}_3$: C, 61.26; H, 3.33; N, 21.01. Found: C, 61.25; H, 3.30; N, 20.99.

3-Phenyl-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (7d). Yield – Method A, 72.9%; method B, 80.7%. M.p. – 246–248°C; $^1\text{H-NMR}$, δ : 7.58 (m, 3H, H-3, H-4, H-5 Ph), 7.83 (dt, 1H, $^3J=8.0$ Hz, $^4J=0.8$ Hz, H-10), 7.95 (d, 1H, $J=8.0$ Hz, H-8), 8.05 (dt, 1H, $^3J=8.2$ Hz, $^4J=1.3$ Hz, H-9), 8.21 (d, 2H, $^3J=8.2$ Hz, $^4J=1.2$ Hz, H-2, H-6 Ph), 8.59 (dd, 1H, $^3J=8.1$ Hz, $^4J=0.8$ Hz, H-11), 9.09 (s, 1H, H-6); $^{13}\text{C NMR}$, δ : 119.8, 125.9, 128.2, 128.7, 129.7, 129.8, 131.7, 132.2, 135.9, 144.2, 144.7, 151.3, 151.7, 160.4; LC-MS, m/z =275 [M+1]; EI-MS, m/z (I_{rel} , %) = 275 (4.7), 274 (2.1), 248 (4.3), 205 (3.3), 172 (9.8), 171 (100.0), 143 (3.7), 129 (14.2), 103 (11.1), 102 (6.2), 77 (3.3), 76 (19.5), 75 (6.3), 74 (2.7), 64 (5.5), 63 (20.3), 62 (11.2), 61 (3.0), 52 (4.9), 51 (5.5), 50 (6.9); Anal. calcd. for $C_{16}\text{H}_{10}\text{N}_4\text{O}$: C, 70.07; H, 3.67; N, 20.43. Found: C, 70.05; H, 3.63; N, 20.45.

3-(4-Methylphenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (7e). Yield – Method A, 83.2%; method B, 80.7%; $^1\text{H-NMR}$, δ : 2.46 (s, 3H, CH_3), 7.28 (d, 2H, $J=8.2$ Hz, H-3, H-5 Ph), 7.77 (t, 1H, $J=7.8$ Hz, H-10), 7.89 (d, 1H, $J=7.9$ Hz, H-8), 7.97 (t, 1H, $J=8.0$ Hz, H-9), 8.22 (d, 2H, $J=8.2$ Hz, H-2, H-6 Ph), 8.67 (d, 1H, $J=8.1$ Hz, H-11), 8.81 (s, 1H, H-6); LC-MS, m/z =289 [M+1]; EI-MS, m/z (I_{rel} , %) = 172 (8.4), 171 (100), 143 (5.5), 129 (50.2), 117 (33.4), 116 (45.7), 103 (5.9), 102 (27.1); Anal. calcd. for $C_{17}\text{H}_{12}\text{N}_4\text{O}$: C, 70.82; H, 4.20; N, 19.43. Found: C, 70.83; H, 4.20; N, 19.43.

3-(4-Methoxyphenyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (7f). Yield – Method A, 49.3%; method B, 86.4%. M.p. – 247–248°C; $^1\text{H-NMR}$, δ : 3.85 (s, 3H, OCH_3), 7.11 (d, 2H, $J=8.2$ Hz, H-3, H-5 Ph), 7.81 (t, 1H, $J=7.8$ Hz, H-10), 7.94 (d, 1H, $J=7.9$ Hz, H-8), 8.03 (t, 1H, $J=7.8$ Hz, H-9), 8.31 (d, 2H, $J=8.2$ Hz, H-2, H-6 Ph), 8.57 (d, 1H, $J=7.8$ Hz, H-11), 9.06 (s, 1H, H-6); LC-MS, m/z =305 [M+1]; EI-MS, m/z (I_{rel} , %) = 304 (3.1), 276 (8.1), 190 (7.4), 187 (5), 172 (8.8), 171 (100), 169 (7.7), 134 (6.6), 133 (58.8), 132 (7.2), 129 (33.9), 111 (5.7), 102 (13.4); Anal. calcd. for $C_{17}\text{H}_{12}\text{N}_4\text{O}_2$: C, 67.10; H, 3.97; N, 18.41. Found: C, 67.13; H, 4.01; N, 18.43.

3-(2-Thienyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (7g). Yield – Method A, 71.4%; method B, 83.0%. M.p. – 279–280°C; $^1\text{H-NMR}$, δ : 7.31 (t, 1H, $J=4.3$ Hz, H-4 Th.), 7.98 (d, 1H, $J=5.0$ Hz, H-3 Th.), 7.82 (t, 1H, $J=7.8$ Hz, H-10), 7.94 (d, 1H, $J=8.1$ Hz, H-8), 8.04 (t, 1H, $J=7.8$ Hz, H-9), 8.40 (d, 1H, $J=3.2$ Hz, H-5 Th.), 8.58 (d, 1H, $J=8.1$ Hz, H-11), 9.04 (s, 1H, H-6); $^{13}\text{C-NMR}$, δ : 120.0, 125.9, 128.3, 128.6, 129.7, 133.0, 133.6, 134.4, 135.8, 144.1, 144.4, 146.7, 151.1, 159.4; LC-MS, m/z =281 [M+1]; EI-MS, m/z (I_{rel} , %): 254 (7.0), 227 (13.6), 213 (12.7), 211 (16.6), 173 (7.6), 172 (88.5), 171 (93.9), 143 (27.7), 130 (13.5), 129 (100.0), 116 (6.1), 110 (5.1), 109 (58.1), 103 (6.2), 102 (35.7); Anal. calcd. for $C_{14}\text{H}_8\text{N}_4\text{OS}$: C, 59.99; H, 2.88; N, 19.99; S, 11.44. Found: C, 60.01; H, 2.91; N, 20.03; S, 11.46.

The general method of synthesis of 3-[2-oxo-2-ethyl]-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (8a-8d).

Heat esters **6a-6d** (0.005 Mol) for 6 h in 10 ml of glacial acetic acid. Remove the solvent under the vacuum, add 5 ml of methanol to the residue, filter the precipitate, wash on the filter with diethyl ether and dry.

3-(2-Phenyl-2-oxoethyl)-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (8a). Yield – 74.4%. M.p. – 286–288°C. $^1\text{H NMR}$, δ : 4.58 (s, 2H, CH_2), 7.54 (t, 2H, $J=7.8$ Hz, H-3',5'), 7.72 (t, 1H, $J=7.8$ Hz, H-4'), 7.82 (t, 1H, $J=7.8$ Hz, H-10), 7.94 (d, 1H, $J=8.0$ Hz, H-8), 8.04 (t, 1H, $J=7.8$ Hz, H-9), 8.09 (d, 2H, $J=8.0$ Hz, H-2',6'), 8.31 (d, 1H, $J=8.0$ Hz, H-11), 8.99 (s, 1H, H-6). $^{13}\text{C NMR}$ (100 MHz) δ = 41.70, 120.11, 125.91, 128.30, 128.89 (2C), 129.34 (2C), 129.62, 134.29, 136.04, 136.49, 144.29, 144.37, 152.32, 155.40, 160.60, 195.41. LC-MS, m/z =317 [M+1]. Anal. calcd for $C_{18}\text{H}_{12}\text{N}_4\text{O}_2$: C, 68.35; H, 3.82; N, 17.71. Found: C, 68.41; H, 3.75; N, 17.79.

3-[2-(4-Methylphenyl)-2-oxoethyl]-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (8c). Yield – 51.5%. M.p. – 236–238°C. $^1\text{H NMR}$, δ : 2.41 (s, 3H, CH_3), 4.54 (s, 2H, CH_2), 7.39, 7.98 (d, 2H, $J=8.0$ Hz, H_{Phenyl}), 7.82 (t, 1H, $J=7.8$ Hz, H-10), 7.94 (d, 1H, $J=8.0$ Hz, H-8), 8.05 (t, 1H, $J=7.8$ Hz, H-9), 8.57 (d, 1H, $J=8.0$ Hz, H-11), 8.99 (s, 1H, H-6). $^{13}\text{C NMR}$ (100 MHz) δ = 21.72, 41.59, 120.15, 125.93, 128.32, 129.01 (2C), 129.63, 129.87 (2C), 134.06, 136.04, 144.33, 144.40, 144.79, 152.34, 155.47, 160.62, 194.87. LC-MS, m/z =331 (MH $^+$). Anal. calcd for $C_{19}\text{H}_{14}\text{N}_4\text{O}_2$: C, 69.08; H, 4.27; N, 16.96. Found: C, 69.19; H, 4.21; N, 16.98.

3-[2-(4-Methoxyphenyl)-2-oxoethyl]-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (8c). Yield – 71.5%. M.p. – 276–278°C. $^1\text{H NMR}$, δ : 3.88 (s, 3H, OCH_3), 4.52 (s, 2H, CH_2), 7.10 (d, 2H, $J=8.1$ Hz, H-3',5'), 7.82 (t, 1H, $J=7.8$ Hz, H-10), 7.94 (d, 1H, $J=8.0$ Hz, H-8), 8.05 (m, 3H, H-9,2',6'), 8.58 (d, 1H, $J=8.0$ Hz, H-11), 8.98 (s, 1H, H-6). $^{13}\text{C NMR}$, δ : 41.38, 56.14, 114.53, 120.15, 125.92, 128.31, 129.45, 129.63, 131.29, 136.05, 144.33, 144.41, 152.32, 155.57, 160.63, 164.09, 193.68. LC-MS, m/z =348, 347 [M+1]. EI-MS, m/z (I_{rel} , %) = 346 (M $^+$, 13.8), 175 (5.2), 172 (6.1), 171 (40.4), 136 (10.9), 135 (100.0), 129 (17.8), 102 (5.2), 92 (5.2). Anal. calcd for $C_{19}\text{H}_{14}\text{N}_4\text{O}_3$: C, 65.89; H, 4.07; N, 16.18. Found: C, 65.95; H, 3.92; N, 16.31.

3-[2-Thiophen-2-yl-2-oxoethyl]-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (8d). Yield – 72.9%. M.p. – 256–258°C. $^1\text{H NMR}$, δ : 4.52 (s, 2H, CH_2), 7.31 (t, 1H, $J=4.0$ Hz, H-4'), 7.79 (t, 1H, $J=7.8$ Hz, H-10), 7.91 (d, 1H, $J=7.9$ Hz, H-8), 8.03 (t, 1H, $J=8.0$ Hz, H-9), 8.09 (d, 1H, $J=4.9$ Hz, H-3'), 8.18 (d, 1H, $J=3.2$ Hz, H-5'), 8.55 (d, 1H, $J=8.0$ Hz, H-11), 8.97 (s, 1H, H-6). $^{13}\text{C NMR}$, δ : 42.02, 120.10, 125.93, 128.29, 129.41, 129.62, 135.23, 136.05, 136.24, 143.36, 144.29, 144.38, 152.33, 154.73, 160.57, 188.00. LC-MS, m/z =323 [M+1]. EI-MS, m/z (I_{rel} , %) = 325 (5.2), 324 ([M + 2] $^{+}$, 13.4), 322 (M $^{+}$,

34.5), 321 (100.0), 170 (13.1), 168 (6.3). Anal. calcd for $C_{16}H_{10}N_4O_2S$: C, 59.62; H, 3.13; N, 17.38. Found: C, 59.70; H, 3.21; N, 17.29.

The general method of synthesis of 3-R-2H-[1,2,4]triazino[2,3-c]quinazoline-2,6(7H)-dione (9a-9d).

Add carbonyldiimidazole (0.0055 Mol) to the suspension of the corresponding 2-R-[2-oxo-2,3-dihydroquinazolin-4 (1*H*)-ylidene]hydrazone]acetic acid (**5.1**, **5.4-5.6**, **5.8**) (0.005 Mol) in 10 ml of anhydrous dioxane or 0.9 g of DMF. Heat the mixture at 80°C for 1 h, cool, filter the precipitate formed and dry.

3-Methyl-2H-[1,2,4]triazino[2,3-c]quinazoline-2,6(7H)-dione (9a). Yield – 78.9%. M.p. >320°C; 1H -NMR, δ : 2.33 (s, 3H, CH_3), 7.50 (m, 2H, H-9, H-8), 7.74 (t, 1H, J =7.6 Hz, H-10), 8.29 (d, 1H, J =8.1 Hz, H-11), 11.97 (s, 1H, 7-NH); LC-MS, m/z =229 [M+1]; Anal. calcd. for $C_{11}H_8N_4O_2$: C, 57.89; H, 3.53; N, 24.55. Found: C, 57.90; H, 3.54; N, 24.57.

3-Phenyl-2H-[1,2,4]triazino[2,3-c]quinazoline-2,6(7H)-dione (9b). Yield – 91.9%. M.p. >320°C; 1H -NMR, δ : 7.29 (d, 1H, J =8.0 Hz, H-8), 7.35 (t, 1H, J =7.8 Hz, H-9), 7.55 (m, 3H, H^3, H^4, H^5 Ph), 7.75 (t, 1H, J =7.4 Hz, H-10), 8.19 (d, 2H, H-2, H-6 Ph, J =7.4 Hz), 8.29 (d, 1H, J =8.0 Hz, H-11), 12.37 (s, 1H, 7-NH); ^{13}C -NMR, δ : 160.4, 154.4, 149.6, 145.3, 138.8, 136.1, 132.7, 131.4, 129.6, 128.7, 126.9, 124.0, 116.0, 113.5; LC-MS, m/z =291 [M+1]; EI-MS, m/z (I_{rel} , %) = 290 (7.7), 263 (6.3), 188 (12.6), 187 (100), 171 (6.3), 159 (30.6), 145 (20.8), 144 (8.8), 117 (9.9), 103 (23.4); Anal. calcd. for $C_{16}H_{10}N_4O_2$: C, 66.20; H, 3.47; N, 19.30. Found: C, 66.23; H, 3.51; N, 19.35.

3-(4-Methylphenyl)-2H-[1,2,4]triazino[2,3-c]quinazoline-2,6(7H)-dione (9c). Yield – 99.5%. M.p. >320°C; 1H -NMR, δ : 2.39 (s, 3H, CH_3), 7.30 (d, 1H, J =8.0 Hz, H-8), 7.35 (t, 1H, H-9), 7.39 (d, 2H, J =8.0 Hz, H-3, H-5 Ph), 7.76 (t, 1H, J =7.8 Hz, H-10), 8.13 (d, 2H, J =8.2 Hz, H-2, H-6 Ph), 8.28 (d, 1H, J =7.8 Hz, H-11), 12.36 (s, 1H, 7-NH); LC-MS, m/z =305 [M+1]; Anal. calcd. for $C_{17}H_{12}N_4O_2$: C, 67.10; H, 3.97; N, 18.41. Found: C, 67.08; H, 3.95; N, 18.39.

3-(4-Methoxyphenyl)-2H-[1,2,4]triazino[2,3-c]quinazoline-2,6(7H)-dione (9d). Yield – 99.5%. M.p. >320°C; 1H -NMR, δ : 3.84 (s, 3H, OCH_3), 7.07 (d, 2H, J =8.9 Hz, H-3, H-5 Ph), 7.30 (d, 1H, J =8.2 Hz, H-8), 7.35 (t, 1H, J =7.4 Hz, H-9), 7.75 (t, 1H, J =7.4 Hz, H-10), 8.27 (m, 3H, H-11 Quin, H-2, H-6 Ph), 12.35 (s, 1H, 7-NH); LC-MS, m/z =321 [M+1]; Anal. calcd. for $C_{17}H_{12}N_4O_3$: C, 63.75; H, 3.78; N, 17.49. Found: C, 63.78; H, 3.81; N, 17.41.

3-(2-Thienyl)-2H-[1,2,4]triazino[2,3-c]quinazoline-2,6(7H)-dione (9e). Yield – 91.2%. M.p. >320°C; 1H -NMR, δ : 7.27 (t, 1H, J =4.9 Hz, H-4 Th.), 7.32 (d, 1H, J =8.0 Hz, H-8), 7.37 (t, 1H, J =7.8 Hz, H-9), 7.78 (t, 1H, J =7.4 Hz, H-10), 7.93 (d, 1H, J =4.5 Hz, H-5 Th.), 8.29 (d, 1H, J =8.1 Hz, H-11), 8.32 (s, 1H, H-3 Th.), 12.43 (s, 1H, 7-NH); LC-MS, m/z =297 [M+1]; Anal. calcd. for

$C_{14}H_8N_4O_2S$: C, 56.75; H, 2.72; N, 18.91; S, 10.82. Found: C, 56.79; H, 2.74; N, 18.94; S, 10.84.

The general method of synthesis of 3-(2-aminophenyl)-6-R-1,2,4-triazin-5(2H)-ones (10a-10g).

Add hydrazine hydrate (0.025 Mol) to the suspension of the corresponding substituted 3-R-2H-[1,2,4]triazino[2,3-c]quinazolin-2-one (**7.1-7.7**) (0.005 Mol) in 15 ml of propan-2-ol and reflux for 1 h. Cool the reaction mixture, acidify with hydrochloric acid to pH=4-5. Filter the precipitate formed, wash with water and dry.

3-(2-Aminophenyl)-6-methyl-1,2,4-triazin-5(2H)-one (10a). Yield – 46.5%. M.p. – 240-241°C; 1H -NMR, δ : 2.19 (s, 3H, CH_3), 6.60 (t, 1H, J =7.9 Hz, H-5), 6.80 (d, 1H, J =8.1 Hz, H-3), 7.25 (t, 1H, J =8.3 Hz, H-4), 7.71 (d, 1H, J =8.0 Hz, H-6), 8.44 (br.s., 2H, -NH₂); ^{13}C -NMR, δ : 17.6, 110.2, 115.7, 115.7, 117.5, 117.5, 128.7, 133.4, 133.4, 150.2; LC-MS, m/z =203 [M+1], Anal. calcd. for $C_{10}H_{10}N_4O$: C, 59.40; H, 4.98; N, 27.71. Found: C, 59.42; H, 4.99; N, 27.74.

3-(2-Aminophenyl)-6-benzyl-1,2,4-triazin-5(2H)-one (10b). Yield – 92.4%. M.p. – 282-284°C; 1H -NMR, δ : 3.93 (d, 1H, J =6.2 Hz, CH_2), 6.59 (t, 1H, J =7.8 Hz, H-5), 6.80 (d, 1H, J =8.0 Hz, H-3), 7.22 (t, 1H, J =6.6 Hz, H-4), 7.30 (m, 5H, H-2', H-3', H-4', H-5', H-6' Ph), 7.60 (d, 1H, J =8.2 Hz, H-6), 9.12 (br.s., 2H, -NH₂); ^{13}C -NMR, δ : 36.7, 109.9, 115.7, 117.5, 126.9, 128.6, 128.7, 128.7, 129.8, 129.8, 129.8, 129.8, 133.5, 137.1, 150.4; LC-MS, m/z =279 [M+1], Anal. calcd for $C_{16}H_{14}N_4O$: C, 69.05; H, 5.07; N, 20.13. Found: C, 69.08; H, 5.09; N, 20.15.

3-(2-Aminophenyl)-6-(4-nitrobenzyl)-1,2,4-triazin-5(2H)-one (10c). Yield – 82.5%. M.p. – 264-266°C; 1H -NMR, δ : 4.10 (s, 1H, CH_2), 6.59 (t, 1H, J =7.4 Hz, H-5 Ph), 6.80 (d, 1H, J =8.4 Hz, H-3 Ph), 7.23 (t, 1H, J =7.2 Hz, H-4 Ph), 7.60 (m, 3H, H-6 Ph, H-2', H-6' Ph), 8.18 (d, 2H, J =8.0 Hz, H-3', H-5' Ph), 9.18 (br.s., 2H, -NH₂); ^{13}C -NMR, δ : 36.03, 109.17, 115.12, 116.97, 123.23, 128.06, 130.63, 132.97, 145.05, 146.18, 149.84; LC-MS, m/z =324 [M+1], Anal. calcd. for $C_{16}H_{13}N_5O_3$: C, 59.44; H, 4.05; N, 21.66.17. Found: C, 59.45; H, 4.06; N, 21.69.

3-(2-Aminophenyl)-6-phenyl-1,2,4-triazin-5(2H)-one (10d). Yield – 58.7%. M.p. – 290-292°C; 1H -NMR, δ : 6.64 (t, J =7.4 Hz, 1H, H-5 Ph), 6.84 (d, 1H, J =8.5 Hz, H-3 Ph), 7.26 (t, 1H, J =7.4 Hz, H-4 Ph), 7.50 (m, 3H, H-3', H-4', H-5' Ph), 7.72 (d, 1H, J =7.8 Hz, H-6 Ph), 8.14 (d, 2H, J =4.1 Hz, H-2', H-6' Ph), 9.5 (br.s., 2H, -NH₂); ^{13}C -NMR, δ : 109.7, 115.7, 117.5, 128.6, 128.6, 128.6, 128.8, 128.8, 128.9, 128.9, 128.9, 130.4, 133.5, 133.6, 150.6; LC-MS, m/z =265 [M+1], Anal. calcd. for $C_{15}H_{12}N_4O$: C, 68.17; H, 4.58; N, 21.20. Found: C, 68.20; H, 4.59; N, 21.24.

3-(2-Aminophenyl)-6-(4-methylphenyl)-1,2,4-triazin-5(2H)-one (10e). Yield – 52.2%. M.p. – 258-260°C; 1H -NMR, δ : 2.36 (s, 3H, CH_3), 6.63 (t, 1H, J =7.4 Hz, H-5), 6.83 (d, 1H, J =8.2 Hz, H-3), 7.26 (t, 1H, J =7.4 Hz, H-4), 7.28 (d, 2H, J =8.2 Hz, H-3', H-5'), 7.69 (d, 1H, J =8.4 Hz,

H-6), 8.06 (d, 2H, $J=8.2$ Hz, H-2',H-6' Ph), 9.17 (br.s., 2H, -NH₂); ¹³C-NMR, δ : 21.01, 109.16, 115.12, 116.95, 128.19, 128.60, 130.12, 132.96, 139.64, 149.92; LC-MS, m/z =279 [M+1], Anal. calcd. for C₁₆H₁₄N₄O: C, 69.05; H, 5.07; N, 20.13. Found: C, 69.09; H, 5.11; N, 20.18.

3-(2-Aminophenyl)-6-(4-methoxyphenyl)-1,2,4-triazin-5(2H)-one (10f) Yield – 74.8%. M.p. – 265–266°C; ¹H-NMR, δ : 3.82 (s, 3H, OCH₃), 6.63 (t, $J=7.8$ Hz, 1H, H-5 Ph), 6.83 (d, $J=8.4$ Hz, 1H, H-3 Ph,), 7.06 (d, $J=8.9$ Hz, 2H, H-3', H-5' Ph), 7.25 (t, $J=8.0$ Hz, 1H, H-4 Ph), 7.70 (d, $J=8.2$ Hz, 1H, H-6 Ph), 8.18 (d, $J=8.8$ Hz, 2H, H-2', H-6' Ph), 9.20 (br.s., 2H, -NH₂); ¹³C-NMR, δ : 55.22, 109.23, 113.47, 115.11, 116.93, 125.23, 128.16, 129.88, 132.89, 149.87, 160.61; Anal. calcd. for C₁₆H₁₄N₄O₂: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.33; H, 4.81; N, 19.11.

3-(2-Aminophenyl)-6-(thienyl-2)-1,2,4-triazin-5(2H)-one (10g). Yield – 25.8%. M.p. – 296–297°C; ¹H-NMR, δ : 6.62 (t, $J=7.8$ Hz, 1H, H-4 Ph), 6.82 (d, $J=8.1$ Hz, 1H, H-6 Ph), 7.25 (t, $J=7.8$ Hz, 1H, H-5 Ph), 7.31 (t, $J=4.2$ Hz, 1H, H-4 Th.), 7.67 (d, $J=7.8$ Hz, 1H, H-3 Ph), 7.78 (d, $J=4.9$ Hz, 1H, H-5 Th.), 8.21 (d, $J=3.4$ Hz, 1H, H-3 Th.), 9.26 (br.s., 2H, -NH₂); ¹³C-NMR, δ : 150.4, 135.7, 133.5, 131.2, 130.6, 130.6, 128.8, 128.8, 128.3, 117.5, 115.8, 109.9; LC-MS, m/z =269 [M+1], Anal. calcd. C₁₃H₁₀N₄OS: C, 57.76; H, 73; N, 20.73. Found: C, 57.76; H, 3.73; N, 20.73.

The general method of synthesis 3'-(2-amino-phenyl)-3-R-spiro[pyrazoline-5,6'(1'H)-1,2,4-triazin-5'(4'H)-one (11.1-11.4).

Add hydrazine hydrate (0.40 Mol) to the appropriate 3-[2-oxo-2-R-ethyl]-2H-[1,2,4]triazino[2,3-c]quinazolin-2-ones (**8a-8d**) (0.10 Mol) in 15 ml of propan-2-ol, reflux the resulting mixture for 6–8 h. After cooling filter the precipitate and wash with propan-2-ol.

3'-(2-Aminophenyl)-3-phenyl-spiro[pyrazoline-5,6'(1'H)-1,2,4-triazin-5'(4'H)-one (11a). Yield – 87.5%. M.p. – 236–238°C. ¹H NMR, δ : 2.95, 3.84 (d, 1H, $J=17.6$ Hz, CH₂), 6.32 (br. s, 2H, NH₂), 6.55 (t, 1H, $J=7.6$ Hz, H-5'), 6.71 (d, 1H, $J=7.8$ Hz, H-3'), 7.05 (t, 1H, $J=7.8$ Hz, H-4'), 7.35 (d, 1H, $J=7.6$ Hz, H-6'), 7.40 (t, 3H, $J=7.6$ Hz, H-3,4,5), 7.65 (d, 2H, $J=7.4$ Hz, H-2,6), 8.22, 8.14 (s, 1H, NH), 10.84 (s, 1H, NH). ¹³C NMR, δ : 39.01, 77.72, 113.16, 115.29, 116.04, 125.64, 125.86, 127.86, 128.77, 129.01, 129.42, 129.87, 133.31, 140.31, 147.59, 148.10, 163.97. LC-MS, m/z =322, 321 [M+1]. Anal. calcd for C₁₇H₁₆N₆O: C, 63.74; H, 5.03; N, 26.23. Found: C, 63.88; H, 5.12; N, 26.11.

3'-(2-Aminophenyl)-3-(4-methylphenyl)-spiro[pyrazoline-5,6'(1'H)-1,2,4-triazin-5'(4'H)-one (11b). Yield – 68.8%. M.p. – 206–208°C. ¹H NMR, δ : 2.92, 3.80 (d, 1H, $J=17.6$ Hz, CH₂), 6.32 (br. s, 2H, NH₂), 6.54 (t, 1H, $J=7.6$ Hz, H-5'), 6.70 (d, 1H, $J=8.1$ Hz, H-3'), 7.04 (t, 1H, $J=7.8$ Hz, H-4'), 7.20 (d, 2H, $J=8.1$ Hz, H-3,5), 7.41 (d, 1H, $J=7.8$ Hz, H-6'), 7.54 (d, 2H, $J=8.1$ Hz, H-2,6), 8.13, 8.10 (s, 1H, NH), 10.82 (s, 1H, NH). ¹³C NMR, δ : 21.43, 39.10, 77.58, 113.13, 115.25, 116.00, 125.85

(2C), 127.83, 129.59 (2C), 129.83, 130.59, 138.24, 140.25, 147.58, 148.23, 164.02. LC-MS, m/z =335 [M+1]. Anal. calcd for C₁₈H₁₈N₆O: C, 64.66; H, 5.43; N, 25.13. Found: C, 64.77; H, 5.51; N, 25.05.

3'-(2-Aminophenyl)-3-(4-methoxyphenyl)-spiro[pyrazoline-5,6'(1'H)-1,2,4-triazin-5'(4'H)-one (11c). Yield – 91.3%. M.p. – 214–216°C. ¹H NMR, δ : 2.91, 3.80 (d, 1H, $J=17.6$ Hz, CH₂), 6.32 (br. s, 2H, NH₂), 6.54 (t, 1H, $J=7.6$ Hz, H-5'), 6.70 (d, 1H, $J=8.1$ Hz, H-3'), 6.95 (d, 2H, $J=8.5$ Hz, H-3,5), 7.04 (t, 1H, $J=7.8$ Hz, H-4'), 7.41 (d, 1H, $J=7.8$ Hz, H-6'), 7.59 (d, 2H, $J=8.5$ Hz, H-2,6), 7.97, 8.11 (s, 1H, NH), 10.81 (s, 1H, NH). ¹³C NMR, δ : 39.28, 55.68, 77.58, 113.16, 114.46 (2C), 115.26, 116.01, 126.00, 127.38 (2C), 127.82, 129.83, 140.25, 147.58, 148.26, 159.96, 164.08. LC-MS, m/z =351 [M+1]; EI-MS, m/z (I_{rel} %) = 351 ([M + 1]⁺, 13.7), 350 (M⁺, 100.0), 344 (9.8), 334 (16.4), 333 (26.8), 332 ([M - H₂O]⁺⁺, 71.1), 331 (27.6), 321 (10.7), 319 (34.8), 200 (22.9), 198 (24.9), 197 (5.9), 159 (16.3). Anal. calcd for C₁₈H₁₈N₆O₂: C, 61.70; H, 5.18; N, 23.99. Found: C, 61.79; H, 5.25; N, 23.85.

3'-(2-Aminophenyl)-3-(thienyl-2)-spiro[pyrazoline-5,6'(1'H)-1,2,4-triazin-5'(4'H)-one (11d). Yield – 68.6%. M.p. – 256–258°C. ¹H NMR, δ : 2.95, 3.83 (d, 1H, $J=17.6$ Hz, CH₂), 6.31 (br. s, 2H, NH₂), 6.53 (t, 1H, $J=7.6$ Hz, H-5'), 6.69 (d, 1H, $J=8.1$ Hz, H-3'), 7.04 (t, 1H, $J=7.8$ Hz, H-4'), 7.08 (dd, 1H, J_{H4-H5} = 5.1 Hz, J_{H4-H3} = 3.6 Hz, H-4), 7.24 (d, 1H, J_{H3-H4} = 3.6 Hz, H-3), 7.40 (d, 1H, $J=7.8$ Hz, H-6'), 7.50 (d, 1H, J_{H5-H4} = 5.1 Hz, H-5), 8.13, 8.16 (s, 1H, NH), 10.84 (s, 1H, NH). ¹³C NMR, δ : 39.53, 77.84, 113.08, 115.26, 116.02, 126.94, 126.97, 127.83, 128.04, 129.87, 136.94, 140.30, 144.55, 147.58, 163.78. LC-MS, m/z =327 [M+1]. Anal. calcd for C₁₅H₁₄N₆OS: C, 55.20; H, 4.32; N, 25.75. Found: C, 55.32; H, 4.44; N, 25.70.

Cytotoxic activity against malignant human tumor cells

The primary anticancer assay was performed at human tumour cell lines panel derived from nine neoplastic diseases in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. The compounds tested were added to the culture at a single concentration (10⁻⁵ M), and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each compound tested were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The cytotoxic and/or growth inhibitory effects of the most active compounds selected were tested *in vitro* against the full panel of about 60 human tumour cell lines at 10-fold dilutions of five concentrations ranging from 10⁻⁴ to 10⁻⁸ M. A 48-h continuous drug exposure protocol was followed and the SRB protein assay was used to estimate cell viability

or growth. Using seven absorbance measurements [time zero, (T_z), control growth in the absence of a drug (C), and test growth in the presence of a drug at the five concentration levels (T_i)], the percentage growth was calculated at each of the drug concentrations levels. The percentage growth inhibition was calculated as:

$$[(T_i - T_z)/(C - T_z)] \times 100 \text{ for concentrations for which } T_i \geq T_z$$

$$[(T_i - T_z)/T_z] \times 100 \text{ for concentrations for which } T_i < T_z$$

Three dose response parameters were calculated for each compound. The growth inhibition of 50% (GI_{50}) was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which was the drug concentration resulting in 50% lower net protein increase in the treated cells (measured by SRB staining) compared to the net protein increase seen in the control cells. The drug concentration resulting in the total growth inhibition (TGI) was calculated from $T_i = T_z$. The LC₅₀ (the concentration of the drug resulting in 50% reduction in the protein measured at the end of the drug treatment compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested. The log GI₅₀, log TGI,

log LC₅₀ were then determined, defined as the mean of the log's of the individual GI₅₀, TGI, LC₅₀ values. The lowest values were obtained with the most sensitive cell lines.

Conclusions

In the present paper the structural optimization of [(2-R-quinazolin-4-ylidene)hydrazone]carboxylic acids and esters has been conducted, and the series of new heterocycles have been synthesized. Forty of the compounds synthesized have been tested and most of them have exhibited the antitumour activity against leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers cell lines. Screening of the anticancer activity *in vitro* has yielded the most active compounds **2c**, **3b** and **3c** in the micromolar concentrations at the GI₅₀ level (log GI₅₀ is -6.43 up to -5.64 for different cell lines). Compound **2c** has a distinctive selectivity against leukemia, **3b** – against leukemia, non-small cell lung cancer, colon and breast cancer, **3c** – against breast cancer. Compounds **2c**, **3b** and **3c** have shown the highest sensitivity against leukemia cell lines CCRF-CEM (log GI₅₀ = -6.10; 6.05; 5.81, respectively), compounds **3b** and **3c** – against breast cancer cell lines HS 578T (log GI₅₀ = -5.83; -6.43, respectively). All the facts mentioned characterise the class of the compounds under study as a promising object for chemical modification aimed at searching new anticancer agents.

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