Accepted Manuscript

Design, synthesis and biological evaluation of novel quinazoline-2,4-diones conjugated with different amino acids as potential chitin synthase inhibitors

Nada A. Noureldin, Hend Kothayer, El-Sayed M. Lashine, Mohamed M. Baraka, Yanrong Huang, Bing Li, Qinggang Ji

PII: S0223-5234(18)30405-7

DOI: 10.1016/j.ejmech.2018.05.001

Reference: EJMECH 10411

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 6 October 2017

Revised Date: 15 April 2018

Accepted Date: 2 May 2018

Please cite this article as: N.A. Noureldin, H. Kothayer, E.-S.M. Lashine, M.M. Baraka, Y. Huang, B. Li, Q. Ji, Design, synthesis and biological evaluation of novel quinazoline-2,4-diones conjugated with different amino acids as potential chitin synthase inhibitors, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.05.001.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

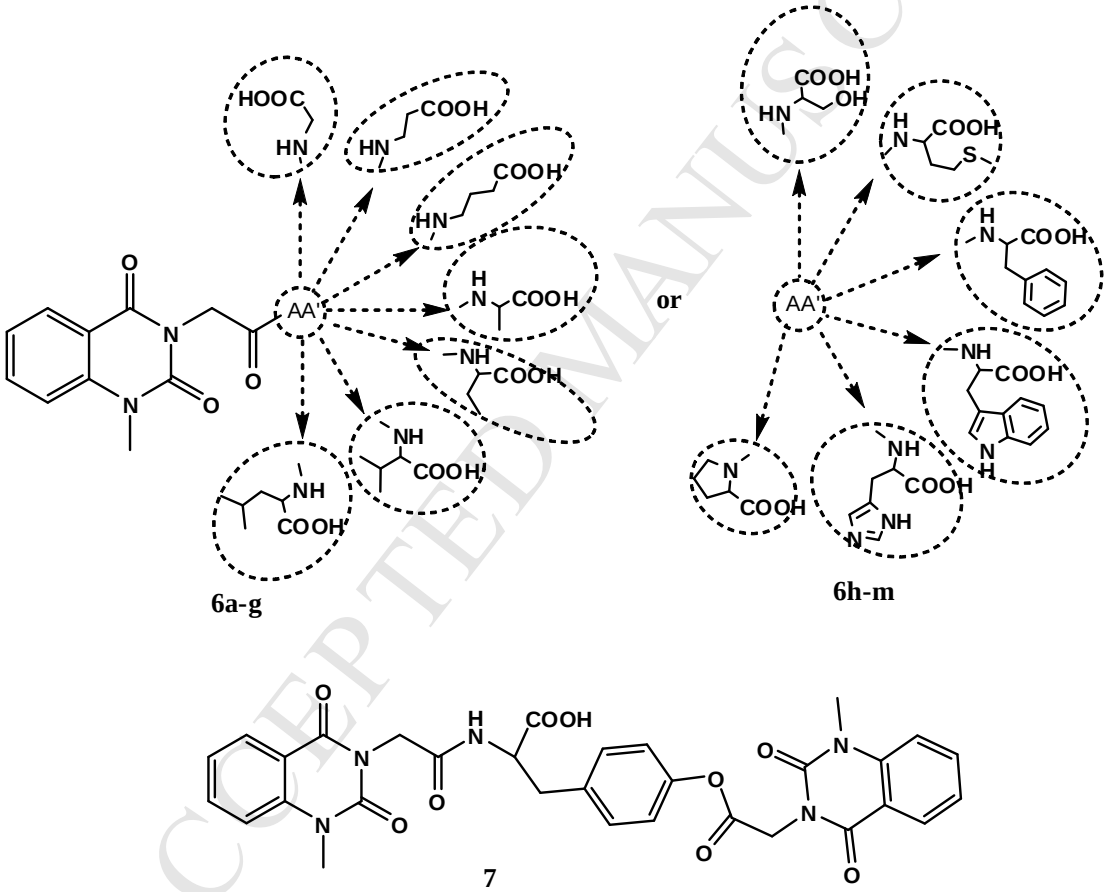
Graphical abstract

Design, Synthesis and Biological Evaluation of Novel Quinazoline-2,4-diones Conjugated with Different Amino Acids as Potential Chitin Synthase Inhibitors

Nada A. Noureldin[a]*, Hend Kothayer[a*1], EL-Sayed M. Lashine[a], Mohamed M. Baraka[a], Yanrong Huang[b], Bing Li[b] and Qinggang Ji[b].

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

[b] *School of chemistry and chemical engineering, Southwest University, P.R.China*



6a-g **6h-m**

7

[1]Corresponding author. Tel. 00201224578259; E-mail: hendo1311@hotmail.com or hkelhamalawy@pharmacy.zu.edu.eg
*equal contribution

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Design, Synthesis and Biological Evaluation of Novel Quinazoline-2,4-diones Conjugated with Different Amino Acids as Potential Chitin Synthase Inhibitors

Nada A. Noureldin[a]*, Hend Kothayer[a*1], EL-Sayed M. Lashine[a], Mohamed M. Baraka[a], Yanrong Huang[b], Bing Li[b] and Qinggang Ji[b].

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

[b] *School of chemistry and chemical engineering, Southwest University, P.R.China*

Abstract: A series of (2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl) acetamido) acids) **(6 a-m), (7)** has been designed to inhibit the action of fungus chitin synthase enzyme (CHS). The synthesis of the designed compounds was carried out in four steps starting from the reaction between 1-methylquinazoline-2,4(1*H*,3*H*)-dione and ethyl chloroacetate to yield the ethyl acetate derivative. This ester was hydrolysed to the corresponding carboxylic acid derivative that was then utilized to couple several amino acids getting the final designed compounds. The synthesised compounds were tested for their inhibition against CHS. Compound **7** showed the highest potency among others with minimum inhibitory concentration (IC_{50}) of 0.166 mmol/L, while polyoxin B (the positive control) had IC_{50} of 0.17 mmol/L. The synthesised compounds were also evaluated for their *in vitro* antifungal activity using *Aspergillus fumigates, Aspergillus flavus, Crytococcus neoformans* and *Candida albicans.* Unfortunately, the 14 synthesized compounds showed lower *in vitro* activity compared to the used active controls. However, compound **6m** and fluconazole have synergistic effect on *Aspergillus flavus*; Compounds **7** and fluconazole have synergistic effects on *Aspergillus fumigates*.

[1]Corresponding author. Tel. 00201224578259; E-mail: hendo1311@hotmail.com or hkelhamalawy@pharmacy.zu.edu.eg
*equal contribution

Keywords: Quinazolinones; Amino acids; Chitin synthase inhibitor; Antifungal.

1. Introduction:

Fungi are eukaryotic organisms with a rigid cell wall containing beta-glucan, chitin and mannoproteins that maintain this rigidity and also take charge of altering host immunity leading to tissue injury and its subsequent events that can even reach to death [1, 2]. Despite the recent medical care advances, invasive fungal infections are still associated with a high mortality rate commonly among immunocompromised patients. The currently available antifungals are considered clinically insignificant concerning their poor bioavailability, resistance development, non-selectivity and serious side effects as nephrotoxicity [3-5]. Thus, continuous development of novel antifungal active agents is needed indeed.

Chitin, the unique fungus cell wall component, considered as one of the recent hopeful antifungal targets [6]. Chitin is a linear polysaccharide composed of *N*-acetyl-D-glucosamine (GlcNAc) units joined by β-1,4-glycoside linkage. When its synthesis is interrupted fungal cell becomes osmotically unstable as it maintains cell wall integrity. Its absence in mammalian cells makes it an excellent target for selective novel antifungal development. Chitin biosynthesis is mediated by chitin synthase (CHS) enzymes that utilize uridine diphosphoryl-*N*-acetyl-D-glucosamine (UDP-GlcNAc) (Fig.1, I) as a substrate for chitin biosynthesis [7, 8].

Till the moment, the exact structure of the active site of the chitin synthase enzyme still unknown as a result of its transmembrane position that hinder the structural studies [9]. The well-known Polyoxins and Nikkomycins are naturally isolated series of compounds which were previously proven for their chitin synthase inhibitory activity, they also possess a core structure similar to that of CHS substrate UDP-GlcNAc [10]. Despite the remarkable *in vitro* activity of

polyoxins against CHS, their limited penetrability across the cell membrane and degradation via intracellular peptidase limit their in vivo activity (Fig.1, II). Their development is now terminated and are limited to be used against phytopathogenic fungi [5]. These drawbacks are not considered in nikkomycins but some of these compounds with hydroxypyridyl residue are unstable in neutral and alkaline pH [7]. Although being tested currently in phase II clinical trials, narrow spectrum of activity of Nikkomycin Z will limit its clinical significance [11, 12] (Fig.1, III).

Fig. 1

Several successful studies were conducted by synthesizing non-nucleoside organic compounds that were then tested for their CHS inhibitory activity, showing promising inhibitory results. Part of these studies involved replacement of the nucleoside part in Nikkomycin Z and polyoxin B by other rings such as quinazoline-2,4-dione (Fig. 2, IV) and coumarin (Fig. 2, V), as a trial to improve drugs hydrophobicity. These rings were designed to be attached with a linear moiety that roughly resemble the side chain of Nikkomycin Z and polyoxin B [13, 14]. Other attempts involved the synthesis of small non-nucleoside organic compounds, many of which showed good inhibitory activity; however; the exact mechanism of action of these compounds still not clear [15-20] (Fig. 3).

Fig. 2

Fig. 3

In the current work, we attempted to design a series of CHS inhibitors that keep the balance between the pharmacokinetics and pharmacodynamics. Whereas, quinazolin-2,4-dione, which already known with its good antifungal action, was used to improve the overall lipophilicity of the compounds in order to improve the cellular penetration to insure their accumulation inside the

fungus cell to the concentration that allow the significant clinical activity [13]. The 3 position in quinazolin-2,4-dione was designed to be attached to a side chain that ends with structurally different amino acids. The side chain has enormous polar groups required for the enzyme's active site interactions. And structurally variant amino acids were used to improve the bioavailability of the target compounds.

Proline, histidine, tryptophan (containing heterocycles), D-alanine, leucine, 2-aminobutyric acid, valine (with alkyl side chain), glycine, β-alanine, GABA (unbranched amino acids), methionine (sulphur containing amino acid), phenylalanine, tyrosine (with aromatic rings) and serine (with terminal hydroxyl group) were used as terminal amino acids to study the structure activity relationship of each change (Fig. 4).

Fig. 4

2. Results and discussion:

2.1. Chemistry:

Compounds **(6a-m)** and **(7)** were synthesized according to Scheme 1. *N*-methyl anthranilic acid **(1)** was fused with urea to yield 1-methylquinazoline-2,4(1*H*,3*H*)-dione **(2)**. Then this intermediate **(2)** was allowed to react with ethyl chloroacetate to form Ethyl 2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl) acetate **(3)** that was then hydrolyzed to the corresponding free carboxylic acid analogue **(4).** This acid was then converted to the acyl benzotriazole **(5)** through reaction with 1H benzotriazole and $SOCl_2$ at room temperature in dry DCM. Finally, this acyl benzotriazole semifinal intermediate **(5)** was allowed to react with various amino acids in acetonitrile/water mixture using triethylamine at room temperature that yielded the novel acetamido acids **(6a-m).** Unexpectedly, when tyrosine (one of the amino acids) was used in such reaction, it yielded the dimer compound **(7)**. This could be explained by the

presence of two reactive nucleophilic centers in tyrosine (the phenolic OH and the NH_2 groups) which both reacted with the highly reactive acyl benzotriazole **(5)**. Unfortunately, our trials to obtain the monomer product from tyrosine were failed.

Scheme. 1

2.2.Biological assays:

2.2.1. Inhibition against yeast CHS:

Compounds **6c, 6l, 6m** and **7** exhibited the most promising enzyme inhibition percent as shown in (Fig. 5, table. 1) at a concentration of 300 μg/ml. Therefore, the IC_{50} values of each of these four compounds were determined where their IC_{50} values ranges from 0.166 mmol/L as that of polyoxin B the reference drug to 0.30 mmol/L. Compound **7** with IC_{50} of 0.166 mmol/L is the strongest inhibitor among the other compounds. While the IC_{50} values for **6c, 6l** and **6m** were 0.30, 0.30 and 0.18 mmol/L respectively (Fig. 6, table. 1).

Fig. 5
Fig. 6
Table. 1

The unexpected dimeric structure of **7** improves the activity in an apparent way over the other monomeric compounds. This result is completely consistent with the two-active site mechanism hypothesis of chitin synthase enzyme, which assumes that CHS has two active sites in close proximity to each other. Thus, dimeric compounds generally possess higher activity than other monomeric ones [7, 21-23].

It is also notable that compounds **6m** and **6l** that carry a terminal nitrogen containing heterocycles like Nikkomycin Z showed the highest inhibitory

activity (IC_{50}= 0.18 and 0.30 mmol/L respectively) among the synthesized monomeric structures.

The superior activity of our unexpected dimeric compound (**7**) not only supports the findings of Finney and Yeager [21, 22] and come against the study of Kral etal. [24] in their debate about the advantage of dimeric chitin synthase inhibitors, but also encourages the designing of future non-nucleoside dimeric chitin synthase inhibitors.

2.2.2. antifungal activity:

Four fungal strains were used to determine the *in vitro* antifungal activity of the synthesized compounds *Aspergillus fumigatus, Aspergillus flavus, Cryptococcus neoformans* and *Candida albicans.* The results are shown in table.2.

The 14 synthesized compounds showed lower *in vitro* activity compared to the two active controls as shown in table. 2. This could be attributed to their failure to penetrate the fungal cell wall and accumulate in the concentration required for the best activity. We may relate this result to the hydrophilicity of the synthesised compounds that hinder their penetration across the fungal cell wall. Future studies are suggested in which the carboxylic acid group could be esterified to improve the lipophilicity of the target compounds and accordingly enhance their penetrability, making a balance between the overall compound lipophilicity and the polar groups required for the active site interactions. Moreover, dimers could be designed and synthesised to make benefit of the two-active site mechanism hypothesis suggested for chitin synthase activity.

Table. 2

*2.2.3. In vitro susceptibility of fungi to **6m**, **7** and fluconazole alone and in combination.*

In order to investigate whether the difficulty in penetration is the only reason of the reduced *in vitro* activity or not, further study was carried out. In this study, the effect of a combination between fluconazole and the most active compounds **6m** and **7** using the checkboard synergy analysis was evaluated using the same four fungal strains used before. Fluconazole was especially used as it acts through inhibition of ergosterol biosynthesis in plasma membrane, leading to disruption in the whole cellular integrity. Thus, it was thought that this may enhance the access of our compounds to their target (the chitin synthase enzyme).

First, the MIC values of **6m**, **7**, and fluconazole were furtherly determined as shown in table. 3. Then the MIC values of both **6m** and **7** in combination with the fluconazole together with the FICIs (Fractional Inhibitory Concentration Index) of each combination against the four fungal strains were calculated. Drug interactions were classified as synergistic if the FIC index was ≤0.5, indifferent if >0.5–4, and antagonistic if >4. From the activity data, compound **6m** and fluconazole have synergistic effect on *Aspergillus flavus* （ATCC 16870); Compounds **7** and fluconazole have synergistic effects on *Aspergillus fumigates* （GIMCC 3.19). The compounds **6m** and **7** have no inhibition or synergistic effect on other fungi. From these results, it is revealed that the failure in penetration is not the only pharmacokinetic factor involved in the reduced *in vitro* activity of the synthesized compounds, however, it is not a negligible factor that if modified the activity will be varied in a positive way.

Table. 3

3. Conclusion:

In summary, chitin synthase enzyme (CHS) is considered as a promising target for selective novel antifungal agents' development. A series of (2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl) acetamido) acids) **(6 a-m),**

ACCEPTED MANUSCRIPT

(7) has been designed, synthesised and evaluated for their inhibition activity against the action of fungus CHS. Four compounds **6c, 6l, 6m** and **7** showed good inhibitory activity. Compound **7** with a dimeric structure was the most active compound with IC_{50}=0.166 mmol/L, while the IC_{50} of Polyoxin B (the reference compound) was 0.17 mmol/L. This result gives rise to the two-active site mechanism hypothesis of chitin synthase enzyme which assume that the enzyme has 2 active sites located in close proximity to each other making dimeric compounds to possess better activity in comparison with monomeric ones. The 14 synthesised compounds were also evaluated for their *in vitro* antifungal activity using four fungal strains *Aspergillus fumigates, Aspergillus flavus, Crytococcus neoformans* and *Candida albicans*. Unfortunately, the 14 synthesized compounds showed lower *in vitro* activity compared to the two active controls (Fluconazole and Polyoxin B). However, compound **6m** and fluconazole have synergistic effect on *Aspergillus flavus*; Compounds **7** and fluconazole have synergistic effects on *Aspergillus fumigates*. Further studies are suggested in which the lipophilicity of the synthesised compounds could be increased through esterification. Also, designing dimeric compounds regarded as a good strategy to obtain more potent CHS inhibitors.

4. Experimental:

4.1. Chemistry:

Melting points (°C) were measured on a Gallenkamp melting point apparatus (London, UK), and are uncorrected. NMR spectra were performed in the Microanalytical Center, Faculty of Pharmacy, Cairo University, Egypt or Microanalytical Center, Faculty of Science, Zagazig University, Egypt. Elemental analyses and Mass spectra were performed in the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt. NMR spectra were recorded on Bruker high performance Digital FT-NMR spectrometer avance III 400 MHz using dimethyl sulfoxide (DMSO)-d_6 and chloroform ($CDCl_3$)-*d* as

solvent and tetramethylsilane (TMS) as an internal standard (chemical shift in δ, ppm). Mass spectra were determined using a GC/MS Shimadzu Qp-2010 plus (Shimadzu Corporation, Tokyo, Japan). Elemental analyses were determined using the Vario EL-III (Elementar) CHNS analyzer (Hanau, Germany). All reactions were monitored by thin layer chromatography (TLC) using silica gel 60 GF245 (E-Merck, Germany) and were visualized by UV-lamp at wavelength (λ) 254 nm.

1-methylquinazoline-2,4(1H,3H)-dione **(2)**, was synthesized according to the previously reported procedures[13]

General method for synthesis of Ethyl2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetate **(3)**:

A mixture of 1-methylquinazoline-2,4(1*H*,3*H*)-dione (**1**, 0.0225 mole), ethyl chloroacetate (0.0225 mole) and K_2CO_3 (0.045mole) in DMF was refluxed for 12 h. After cooling the mixture poured into excess amount of water with stirring. The formed precipitate was filtered washed several times with water to provide ethyl 2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl) acetate **(3)** in 87.14% yield and sufficient purity to be used in the next step without further purification.

Ethyl2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetate **(3)**: (87.14% yield). M.p. 124-127°C. ^{1}H NMR (DMSO, d_6) δ 1.22 (3H, t, *J*= 7.1 Hz, $CH_2\underline{CH_3}$), 3.56 (3H, s, $N\underline{CH_3}$), 4.14 (2H, q, *J*= 7.1 Hz, $\underline{CH_2}CH_3$), 4.71 (2H, s, $N\underline{CH_2}$), 7.36 (1H, t, *J*= 7.5 Hz, H-6), 7.52 (1H, d, *J*= 8.4 Hz, H-8), 7.84 (1H, t, *J*= 7.07 Hz, H-7), 8.08 (1H, d, *J*= 7.8 Hz, H-5).

General method for synthesis of 2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl) acetic acid **(4)**:

A solution of NaOH (0.115 mole) in least amount of water (2ml) was added to a solution of ethyl 2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-

3(4*H*)-yl) acetate (**3**, 0.0115 mole) in methanol (50 ml). The mixture was refluxed for 2 h, then cooled and acidified with 3N HCl to pH~ 5. The formed precipitate was filtered and washed with water and dried to obtain 2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl) acetic acid **(4)** in 62.5% yield in sufficient purity to be used in the next step.

2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl) acetic acid **(4)**: (62.50% yield). M. p. 240-243 °C. ^{1}H NMR (DMSO, d_6) δ 3.55 (3H, s, N$\underline{CH_3}$), 4.63 (2H, s, N$\underline{CH_2}$), 7.35 (1H, t, *J*= 7.6 Hz, H-6), 7.52 (1H, d, *J*= 8.4 Hz, H-8), 7.83 (1H, t, *J*= 8.4 Hz, H-7), 8.08 (1H, d, *J*= 7.8 Hz, H-5), 13.06 (1H, s, COO$\underline{H}$).

General method for synthesis of 3-(2-(1H-benzo[d][1,2,3]triazol-1-yl)-2-oxoethyl)-1-methylquinazoline-2,4(1H,3H)-dione **(5)**:

To a solution of 1H-benzotriazole (0.032 mole) in anhydrous dichloromethane, thionylchloride (0.013 mole) was added and the reaction mixture was stirred at room temperature for 30 min, then the 2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl) acetic acid (**4**, 0.0108 mole) was added and the reaction mixture stirred at room temperature for further 4 h. The precipitate formed was filtered off and the organic layer was extracted twice with 5% Na_2CO_3 solution, washed with water and dried over anhydrous sodium sulphate then the solvent was removed in vacuum to obtain the desired 3-(2-(1*H*-benzo [d] [1,2,3] triazol-1-yl) -2-oxoethyl) -1-methylquinazoline-2,4 (1*H*,3*H*)-dione **(5)** in 82.2 % yield (184-6°C m. p.) and sufficient purity for use in the next step.

3-(2-(1H-benzo[d][1,2,3]triazol-1-yl)-2-oxoethyl)-1-methylquinazoline-2,4(1H,3H)-dione **(5)**: (82.20% yield). M.p. 184-186°C. ^{1}H NMR ($CDCl_3$) δ 3.69 (3H, s, N$\underline{CH_3}$), 5.32 (2H, s, N$\underline{CH_2}$), 7.31-7.37 (2H, m, ArH), 7.56 (1H, t, *J*= 7.7 Hz, ArH), 7.69 (1H, t, *J*= 7.7 Hz, ArH), 7.78 (1H, t, *J*= 7.8 Hz, ArH), 8.20 (1H, d, *J*= 8.2 Hz, ArH), 8.26 (1H, d, *J*= 8.2 Hz, ArH), 8.29 (1H, dd, *J*=7.9 , 1.6 Hz, ArH).

General method for synthesis of (2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl) acetamido) acids) **(6 a-m), (7)**:

A solution of *N*-acylbenzotriazole derivative (**5**, 0.005 mole) and the appropriate amino acid (0.005 mole) in acetonitrile/water mixture (3:1) containing triethylamine (0.0075 mole) was stirred at room temperature for 24 h, then acetonitrile was evaporated under reduced pressure and the residual water solution was then acidified with 1N HCl to pH~5 and the resultant precipitate was then filtered, dried and crystalized from methanol to give the titled compounds (**6 a-m** and **7**) in 32.18-78.47 % yield.

2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido)acetic acid **(6a):** (32.18% yield). M.p. 250-253°C. ^{1}H NMR (DMSO, d_6) δ 3.53 (3H, s, NCH_3), 3.79 (2H, d, *J*= 6 Hz, $NH\underline{CH_2}$), 4.61 (2H, s, NCH_2), 7.34 (1H, t, *J*= 7.6 Hz, H-6), 7.51 (1H, d, *J*= 8.4 Hz, H-8), 7.82 (1H, t, *J*= 7.2 Hz, H-7), 8.05 (1H, dd, *J*= 7.6 Hz, H-5), 8.49 (1H, t, *J*= 5.6 Hz, CONH), 12.60 (1H, bs, COOH). ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 30.85 (NCH_3), 41.13 ($NHCH_2$), 43.76 (NCH_2), 114.04 (ArCH), 115.29 (ArCH), 122.93 (ArCH), 128.56 (ArCH), 135.42 (ArC), 140.69 (ArC), 150.68 (ArC), 161.41 (ArC), 167.51 (ArC), 171.34 (ArC). **MS**, m/z: 291 (M^+). Analysis calcd. for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.52; H, 4.62; N, 14.73.

3-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido)propanoic acid **(6b)**: (76.92% yield). M.p. 231-233 °C.^{1}H NMR (DMSO, d_6) δ 2.39 (2H, t, *J*= 6.8 Hz, CH_2CO), 3.27 (2H, t, *J*= 6.8 Hz, $N\underline{CH_2}CH_2CO$), 3.53 (3H, s, NCH_3), 4.52 (2H, 2H, s, NCH_2), 7.34 (1H, t, *J*= 7.2 Hz, H-6), 7.51 (1H, d, *J*= 8.4 Hz, H-8), 7.82 (1H, t, *J*= 7.2 Hz, H-7), 8.05 (1H, dd, *J*= 8 Hz, H-5), 8.22 (1H, t, *J*= 5.6 Hz, CONH), 12.24 (1H, s, COOH), ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 30.86 (NCH_3), 34.14 ($\underline{C}H_2CO$), 35.27 ($N\underline{C}H_2CH_2CO$), 43.89 (NCH_2), 114.17

(ArCH), 115.33 (ArCH), 122.92 (ArCH), 128.48 (ArCH), 135.43 (ArC), 140.73 (ArC), 150.68 (ArC), 161.40 (ArC), 167.19 (ArC), 173.37 (ArC). **MS**, m/z: 306 (M^+). Analysis calcd. for $C_{14}H_{15}N_3O_5$: C, 55.08; H, 4.95; N, 13.76. Found: C, 55.47; H, 5.03; N, 14.03.

4-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido)butanoic acid **(6c):** (75.79% yield). M.p. 273-275°C. ^{1}H NMR (DMSO, d_6) δ 1.60-1.66 (2H, m, $NHCH_2\underline{CH_2}CH_2$), 2.23 (2H, t, *J*= 7.6 Hz, $NHCH_2CH_2\underline{CH_2}$), 3.05-3.10 (2H, m, $NH\underline{CH_2}CH_2CH_2$), 3.54 (3H, s, NCH_3), 4.52 (2H, s, NCH_2), 7.34 (1H, t, *J*= 7.6 Hz, H-6), 7.49 (1H, d, *J*= 8.4 Hz, H-8), 7.82 (1H, t, *J*= 7.2 Hz, H-7), 8.05 (1H, dd, *J*= 8 Hz, H-5), 8.12 (1H, t, *J*= 6.5 Hz, CONH), 12.05 (1H, bs, COOH), ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 24.81 ($NHCH_2\underline{C}H_2CH_2$), 30.85 ($NCH_3$), 31.44 ($NHCH_2CH_2\underline{C}H_2$), 38.63 ($NH\underline{C}H_2CH_2CH_2$), 43.99 ($NCH_2$), 114.02 (ArCH), 115.41 (ArCH), 122.89 (ArCH), 128.55 (ArCH), 135.37 (ArC), 140.73 (ArC), 150.64 (ArC), 161.49 (ArC), 167.18 (ArC), 174.87 (ArC). **MS**, m/z: 319 (M+). Analysis calcd. for $C_{15}H_{17}N_3O_5$: C, 56.42; H, 5.37; N, 13.16. Found: C, 56.90; H, 5.50; N, 13.42.

2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido)propanoic acid **(6d):** (54.95% yield). M.p. 245-247°C. ^{1}H NMR (DMSO, d_6) δ 1.29 (3H, d, *J*= 7.6, $CH\underline{CH_3}$), 3.53 (3H, s, NCH_3), 4.19-4.27 (1H, m, $\underline{CH}CH_3$), 4.53-4.65 (2H, m, NCH_2), 7.34 (1H, t, *J*= 7.2 Hz, H-6), 7.50 (1H, d, *J*= 8.8 Hz, H-8), 7.82 (1H, t, *J*= 7.2 Hz, H-7), 8.05 (1H, dd, *J*= 8 Hz, H-5), 8.50 (1H, d, *J*= 7.2 Hz, CONH), 12.63 (1H, bs, COOH). ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 18.15 ($CH\underline{C}H_3$), 30.83 (NCH_3), 43.77 (NCH_2), 48.08 ($\underline{C}HCH_3$), 113.88 (ArCH), 115.25 (ArCH), 122.92 (ArCH), 128.65 (ArCH), 135.37 (ArC), 140.62 (ArC), 150.71 (ArC), 161.45 (ArC), 166.75 (ArC), 174.47 (ArC). **MS**, m/z: 306 (M+). Analysis calcd. for $C_{14}H_{15}N_3O_5$: C, 55.08; H, 4.95; N, 13.76. Found: C, 54.96; H, 5.11; N, 14.09.

2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido)butanoic acid **(6e):** (71.49% yield). M.p. 258-259°C. ^{1}H NMR (DMSO, d_6) δ 0.90 (3H, t, *J*= 7.4, $CH_2\underline{CH_3}$), 1.56-1.67 (1H, m, $\underline{CH_2}CH_3$), 1.71-1.78 (1H, m, $\underline{CH_2}CH_3$), 3.53 (1H, s, NCH_3), 4.14-4.20 (1H, m, NH$\underline{CH}$CO), 4.57-4.67 (2H, m, NCH_2), 7.33 (1H, t, *J*= 7.2 Hz, H-6), 7.50 (1H, d, *J*= 8.8 Hz, H-8), 7.82 (1H, t, *J*= 7.2 Hz, H-7), 8.05 (1H, dd, *J*= 8 Hz, H-5), 8.44 (1H, d, *J*= 8 Hz, CONH), 12.62 (1H, bs, COOH). ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 9.97 ($CH_2\underline{C}H_3$), 25.43 ($\underline{C}H_2CH_3$), 30.82 (NCH_3), 43.78 (NCH_2), 53.51 (NH$\underline{C}$HCO), 113.86 (ArCH), 115.28 (ArCH), 122.90 (ArCH), 128.66 (ArCH), 135.35 (ArC), 140.64 (ArC), 150.72 (ArC), 161.45 (ArC), 166.97 (ArC), 173.83 (ArC). **MS**, m/z: 321 (M^+). Analysis calcd. for $C_{15}H_{17}N_3O_5$: C, 56.42; H, 5.37; N, 13.16. Found: C, 56.79; H, 5.23 N, 13.58.

3-methyl-2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido) butanoic acid **(6f)**: (78.47% yield). M.p. 241-243°C.^{1}H NMR (DMSO, d_6) δ 0.90 (6H, dd, *J*= 6.8 Hz, $CH(\underline{CH_3})_2$), 2.02-2.09 (1H, m, $\underline{CH}(CH_3)_2$), 3.53 (3H, s, NCH_3), 4.16-4.20 (1H, m, NH$\underline{CH}$CO), 4.61-4.70 (2H, m, NCH_2), 7.33 (1H, t, *J*= 7.6 Hz, H-6), 7.50 (1H, d, *J*= 8.4 Hz, H-8), 7.82 (1H, t, *J*= 7.2 Hz, H-7), 8.05 (1H, dd, *J*= 7.6 Hz, H-5), 8.40 (1H, d, *J*= 8.4 Hz, CONH), 12.68 (1H, bs, COOH), ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 17.97 ($CH(\underline{C}H_3)_2$), 19.22 ($CH(\underline{C}H_3)_2$), 30.81 (NCH_3), 31.07 ($\underline{C}H(CH_3)_2$), 43.78 (NCH_2), 57.40 (NH$\underline{C}$HCO), 113.85 (ArCH), 115.27 (ArCH), 122.88 (ArCH), 128.66 (ArCH), 135.33 (ArC), 140.64 (ArC), 150.72 (ArC), 161.45 (ArC), 167.16 (ArC), 173.41 (ArC). **MS**, m/z: 334 (M^+). Analysis calcd. for $C_{16}H_{19}N_3O_5$: C, 57.65; H, 5.75; N, 12.61. Found: C, 57.22; H, 5.68; N, 12.97.

4-methyl-2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido) pentanoic acid **(6g):** (39.22% yield). M.p. 213-215°C. ^{1}H NMR (DMSO, d_6) δ 0.86 (3H, d, *J*= 6.5, $CH(\underline{CH_3})_2$), 0.92 (3H, d, *J*= 6.6 Hz, $CH(\underline{CH_3})_2$), 1.52 (2H, t, *J*= 7.6 Hz, $CH\underline{CH_2}CH$), 1.63-1.69 (1H, m, $\underline{CH}(CH_3)_2$), 3.53 (3H, s, NCH_3),

4.22-4.28 (1H, m, NHCHCO), 4.55-4.64 (2H, m, NCH_2), 7.33 (1H, t, *J*= 7.6 Hz, H-6), 7.50 (1H, d, *J*= 8.4 Hz, H-8), 7.82 (1H, t, *J*= 8.4 Hz, H-7), 8.05 (1H, dd, *J*= 8 Hz, H-5), 8.43 (1H, d, *J*= 8 Hz, CONH), 12.54 (1H, bs, COOH). ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 21.93 ($CH(\underline{C}H_3)_2$), 23.04 ($CH(\underline{C}H_3)_2$), 24.68 ($\underline{C}H(CH_3)_2$), 30.83 (NCH_3), 41.22 ($CH\underline{C}H_2CH$), 43.70 (NCH_2), 50.83 (NH$\underline{C}$HCO), 113.97 (ArCH), 115.30 (ArCH), 122.88 (ArCH), 128.58 (ArCH), 135.36 (ArC), 140.68 (ArC), 150.68 (ArC), 161.41 (ArC), 167.03 (ArC), 174.49 (ArC). **MS**, m/z: 348 (M^+). Analysis calcd. for $C_{17}H_{21}N_3O_5$: C, 58.70; H, 6.09; N, 12.10. Found: C, 59.01; H, 6.21; N, 12.37.

3-hydroxy-2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido) propanoic acid **(6h):** (38.23% yield). M.p. 227-230°C.^{1}H NMR (DMSO, d_6) δ 3.53 (3H, s, NCH_3), 3.60-3.64 (1H, m, $\underline{CH_2}OH$), 3.69-3.73 (1H, m, $\underline{CH_2}OH$), 4.28-4.32 (1H, m, NHCHCO), 4.61-4.71 (2H, m, NCH_2), 7.34 (1H, t, *J*= 7.6 Hz, H-8), 7.48 (1H, d, *J*= 8.4 Hz, H-6), 7.82 (1H, t, *J*= 7.2 Hz, H-7), 8.05 (1H, dd, *J*= 7.6 Hz, H-5), 8.42 (1H, d, *J*= 7.6 Hz, CONH), 12.59 (1H, s, COOH), ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 30.85 (NCH_3), 43.84 (NCH_2), 54.98 (NH$\underline{C}$HCO), 62.39 (CH_2OH), 114.06 (ArCH), 115.25 (ArCH), 122.97 (ArCH), 128.58 (ArCH), 135.44 (ArC), 140.69 (ArC), 150.71 (ArC), 161.41 (ArC), 167.13 (ArC), 174.14 (ArC). **MS**, m/z: 321 (M^+). Analysis calcd. for $C_{14}H_{15}N_3O_6$: C, 52.34; H, 4.71; N, 13.08. Found: C, 52.57; H, 4.84; N, 13.29.

2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido)-4-(methylthio)butanoic acid **(6i):** (41.82% yield). M.p. 229-231°C.^{1}H NMR (DMSO, d_6) δ 1.80-1.91 (1H, m, $CH\underline{CH_2}$), 1.95-1.98 (1H, m, $CH\underline{CH_2}$), 2.05 (3H, s, SCH_3), 2.51 (2H, t, *J*= 6 Hz, $CH_2\underline{CH_2}S$), 3.54 (3H, S, NCH_3), 4.33-4.38 (1H, m, NHCHCO), 4.60 (2H, s, NCH_2), 7.34 (1H, t, *J*= 7.6 Hz, H-6), 7.51 (1H, d, *J*= 8.4 Hz, H-8), 7.82 (1H, t, *J*= 8.4 Hz, H-7), 8.05 (1H, d, *J*= 7.6 Hz, H-5), 8.47 (1H, d, *J*= 8 Hz, CONH), 12.72 (1H, bs, COOH), ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 15.30 (SCH_3), 30.09 ($CH_2\underline{C}H_2S$), 30.82 (NCH_3), 31.84 ($CH\underline{C}H_2$),

43.83 (NCH_2), 51.52 (NHCHCO), 113.89 (ArCH), 115.30 (ArCH), 122.90 (ArCH), 128.63 (ArCH), 135.36 (ArC), 140.65 (ArC), 150.69 (ArC), 161.44 (ArC), 167.20 (ArC), 173.44 (ArC). **MS**, m/z: 365 (M^+). Analysis calcd. for $C_{16}H_{19}N_3O_5S$: C, 52.59; H, 5.24; N, 11.50. Found: C, 52.41; H, 5.36; N, 11.54.

2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido)-3-phenylpropanoic acid **(6j):** (65.45% yield). M.p. 238-241°C. ^{1}H NMR (DMSO, d_6) δ 2.89-2.94 (1H, m, $CHCH_2$), 3.01-3.06 (1H, m, $CHCH_2$), 3.53 (3H, s, NCH_3), 4.39-4.44 (1H, m, CH-CH_2), 4.54-4.62 (2H, m, NCH_2), 7.25 (3H, d, *J*= 7.2 Hz, ArH), 7.28-7.35 (3H, m, ArH), 7.50 (1H, d, *J*= 8 Hz, H-8), 7.81 (1H, t, *J*= 7.2 Hz, H-7), 8.04 (1H, dd, *J*= 7.6 Hz, H-5), 8.54 (1H, d, *J*= 8 Hz, CONH), 12.77 (1H, s, COOH). ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 30.83 (NCH_3), 37.46 ($CHCH_2$), 43.84 (NCH_2), 53.70 ($NHCHCH_2$), 113.91 (ArCH), 115.27 (ArCH), 122.92 (ArCH), 126.63 (ArCH), 128.23 (ArCH), 128.68 (ArCH), 129.62 (ArCH), 135.38 (ArC), 136.85 (ArC), 140.64 (ArC), 150.68 (ArC), 161.41 (ArC), 166.90 (ArC), 172.88 (ArC). **MS**, m/z: 381 (M^+). Analysis calcd. for $C_{20}H_{19}N_3O_5$: C, 62.99; H, 5.02; N, 11.02. Found: C, 62.56; H, 4.70; N, 11.21.

3-(1H-indol-3-yl)-2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl) acetamido)propanoic acid **(6k):** (69.84% yield). M.p. 237-239°C.^{1}H NMR (DMSO, d_6) δ 3.03-3.08 (1H, m, $CHCH_2$), 3.15-3.20 (1H, m, $CHCH_2$), 3.54 (3H, s, NCH_3), 4.47-4.52 (1H, m, NHCHCO), 4.57-4.66 (2H, m, NCH_2), 6.99 (1H, t, *J*= 7.6 Hz, ArH), 7.07 (1H, t, *J*= 8 Hz, ArH), 7.17 (1H, d, *J*= 2.4 Hz, CHNH), 7.34 (2H, t, *J*= 7.6 Hz, ArH), 7.49-7.55 (2H, m, ArH), 7.82 (1H, t, *J*= 8.4 Hz, H-7), 8.06 (1H, dd, *J*= 7.6 Hz, H-5), 8.51 (1H, d, *J*= 7.6 Hz, CONH), 10.90 (1H, s, CHNH), 12.70 (1H, bs, COOH). ^{13}C NMR (DMSO, d_6 + $CDCl_3$, *d*) δ 27.48 ($CHCH_2$), 30.83 (NCH_3), 43.91 (NCH_2), 53.33 (NHCHCO), 109.51 (ArCH), 111.38 (ArCH), 113.92 (ArCH), 115.26 (ArCH), 118.69 (ArCH), 118.84 (ArCH), 121.21 (ArCH), 122.91 (ArCH), 123.95 (ArCH), 127.81 (ArC), 128.65 (ArC), 135.36 (ArC), 136.31 (ArC), 140.64 (ArC), 150.71 (ArC), 161.44

(ArC), 166.87 (ArC), 173.40 (ArC). **MS**, m/z: 420 (M^+). Analysis calcd. for $C_{22}H_{20}N_4O_5$: C, 62.85; H, 4.79; N, 13.33. Found: C, 63.13; H, 4.68; N, 13.52.

3-(1H-imidazol-5-yl)-2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido)propanoic acid **(6l):** (43.63% yield). M.p. 185-188°C. ^{1}H NMR (DMSO, d_6) δ 3.06-3.12 (1H, m, CH$\underline{CH_2}$), 3.21-3.26 (1H, m, CH$\underline{CH_2}$), 3.52 (3H, s, NCH_3), 4.63-4.71 (3H, m, NH$\underline{CH}$CO+ NCH_2), 7.19-7.25 (3H, m, H-6 + H-8 +C$\underline{CH}$N), 7.66 (1H, t, *J*= 6.8 Hz, H-5), 8.05 (1H, dd, *J*= 7.6 Hz, H-5), 8.52 (1H, d, *J*= 8 Hz, CONH), 8.66 (1H, d, *J*= 6.8 Hz, NH$\underline{CH}$N), 11.19 (1H, s, COOH), 14.42 (1H, bs, $\underline{NH}$CHN), ^{13}C NMR (DMSO, *d6*) δ 26.77 (NCH_3), 30.92 (CH$\underline{C}H_2$), 43.86 (N$\underline{C}H_2$), 51.62 ($\underline{C}HCH_2$), 114.08 (ArCH), 115.23 (ArCH), 117.16 (ArCH), 122.99 (ArCH), 128.55 (ArCH), 129.45 (ArCH), 132.91 (ArC), 135.47 (ArC), 140.64 (ArC), 150.63 (ArC), 161.39 (ArC), 167.48 (ArC), 171.91 (ArC). **MS**, m/z: 371 (M^+). Analysis calcd. for $C_{17}H_{17}N_5O_5$: C, 54.98; H, 4.61; N, 18.86. Found: C, 55.12; H, 4.73; N, 18.49.

1-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetyl)pyrrolidine-2-carboxylic acid **(6m):** (34.69% yield). M.p. 217-219°C. ^{1}H NMR (DMSO, d_6) δ 1.86-1.93 (1H, m, $NCHCH_2\underline{CH_2}CH_2$), 1.98-2.03 (2H, m, $NCH\underline{CH_2}CH_2CH_2$), 2.15-2.18 (1H, m, $NCHCH_2\underline{CH_2}CH_2$), 3.54 (1H, s, NCH_3), 3.64-3.74 (2H, m, $NCHCH_2CH_2\underline{CH_2}$), 4.24 (1H, dd, *J*= 8.8 Hz, N$\underline{CH}CH_2CH_2CH_2$), 4.66-4.90 (2H, m, NCH_2CO), 7.34 (1H, t, *J*= 7.5 Hz, H-6), 7.49 (1H, d, *J*= 8.4 Hz, H-8), 7.83 (1H, t, *J*= 7.2 Hz, H-7), 8.05 (1H, dd, *J*= 7.8 Hz, H-5), 12.51 (1H, bs, COOH), ^{13}C NMR ($CDCl_3$, *d*) δ 24.84 ($NCHCH_2\underline{C}H_2CH_2$), 27.76 ($NCH\underline{C}H_2CH_2CH_2$), 30.96 ($N\underline{C}H_3$), 42.95 ($N\underline{C}H_2$), 46.85 ($NCHCH_2CH_2\underline{C}H_2$), 60.00 ($N\underline{C}HCH_2CH_2CH_2$), 113.75 (ArCH), 115.20 (ArCH), 123.19 (ArCH), 129.19 (ArCH), 135.49 (ArC), 140.63 (ArC), 150.79 (ArC), 161.58 (ArC), 167.81 (ArC), 173.09 (ArC). **MS**, m/z: 331 (M^+). Analysis calcd. for $C_{16}H_{17}N_3O_5$: C, 58.00; H, 5.17; N, 12.68. Found: C, 58.34; H, 5.54; N, 12.63.

2-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetamido)-3-(4-(2-(1-methyl-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetoxy)phenyl)propanoic acid **(7):** (34.49% yield). M.p. 250-253°C. ^{1}H NMR (DMSO, d_6) δ 2.90-2.96 (1H, m, NHCH$\underline{CH_2}$), 3.02-3.06 (1H, m, NHCH$\underline{CH_2}$), 3.55 (3H, s, NCH_3), 3.59 (3H, s, NCH_3), 4.30-4.43 (1H, m, NH$\underline{CH}CH_2$), 4.58 (2H, s, N$\underline{CH_2}$CONH), 5.01 (2H, s, N$\underline{CH_2}$COO), 7.08 (2H, d, *J*= 8.4 Hz, ArH), 7.28-7.40 (4H, m, ArH), 7.49 (1H, d, *J*= 8.8 Hz, ArH), 7.55 (1H, d, *J*= 8.4 Hz, ArH), 7.81 (1H, t, *J*= 8.8 Hz, ArH), 7.86 (1H, t, *J*= 7.2 Hz, ArH), 8.06 (1H, dd, *J*= 8.6 Hz, ArH), 8.13 (1H, dd, *J*= 7.6 Hz, ArH), 8.53 (1H, d, *J*= 8 Hz, CONH), 12.83 (1H, bs, COOH). ^{13}C NMR (DMSO, *d6*) δ 31.08 (NCH_3), 31.26 (NCH_3), 36.54 (NHCH$\underline{C}H_2$), 43.13 (N$\underline{C}H_2$COO), 43.73 (N$\underline{C}H_2$CONH), 54.07 (NH$\underline{C}HCH_2$), 114.79 (ArCH), 115.09 (ArCH), 115.45 (ArCH), 121.58 (ArCH), 123.31 (ArCH), 123.70 (ArCH), 128.31 (ArCH), 128.38 (ArCH), 130.90 (ArCH), 135.80 (ArCH), 135.93 (ArCH), 136.37 (ArCH), 140.85 (ArC), 149.21 (ArC), 150.50 (ArC), 150.62 (ArC), 161.28 (ArC), 161.34 (ArC), 167.03 (ArC), 167.51 (ArC), 172.97 (ArC). **MS**, m/z: 613 (M^+). Analysis calcd. for $C_{31}H_{27}N_5O_9$: C, 60.68; H, 4.44; N, 11.41. Found: C, 60.89; H, 4.75; N, 11.84.

4.2. Biology:

4.2.1. Extraction of chitin synthase [25]*:*

A single colony of *Saccharomyces cerevisiae* (CGMCC2.145) was inserted into of YPAD liquid medium, and cultured at 30°C for 48 h with oscillating at 65 rpm. The yeast cells were collected through centrifugation at 1500 g for 15 min at 4 °C and discard the supernatant. The pellet was lysed with sonication treatment by being suspended in 20 mL 100mM Tris-HCl media at pH7.0 which contained 40μL fungal protease inhibitor cocktail and 50μL solution of 200mM Phenylmethanesulfonyl fluoride. Cell wall and fragment were removed by centrifugation at 1500 g for 15 min at 4°C and the supernatant was carefully aspirated and placed in a 2 volume of 10% (w / w) sucrose in 100 mM Tris–HCl

at pH 7.5 buffer and then centrifuged at 55000 g for 2 h at 4 °C. After centrifugation, the pellet was re-suspended in 1.6 mL/g Cytoplasmic matrix (50 mM Tris–HCl at pH 7.0 and 33% glycerol) to serve as CHS sample stock solution which could be diluted with 50 mM Tris-HCl buffer at pH 7.5 to get a series of enzyme diluent (1:1, 1:2, 1:4, 1:8, 1:16).

4.2.2. Determination the activity of Chitin Synthase:

200 μL 50 μg/mL WGA buffer solutions in 50 mM Tris–HCl at pH 7.5 was added to each microtiter plate of 96-well plates and oscillation cultured at 37 °C for 2 h. The plates were flushed and washed three times with 50 mM Tris-HCl buffer at pH 7.5, and then 300μL of 10 mg/mL borine serum albumin (50 mM Tris-HCl at pH7.5) buffer solution was added, oscillation incubated for 2 h at 37 °C, which could be stored at -20°C. The plates were emptied and washed at least three times with 50 mM Tris–HCl buffer at pH 7.5. Reaction substrate (100 μL ,40 mM GlcNAc, 2mM UDP–GlcNAc, in 0.1M Tris-Maleic acid buffer at pH 6.8), 50μL CHS diluent and 50 μL of 0.1M Tris- Maleic acid buffer of pH 6.8 were added to appropriate wells. The plates were incubated at 37 °C for 1 h. The wells were emptied and washed three times with 50 mM Tris–HCl buffer at pH 7.5. Each well was added 200 μL of 50 mM pH 7.5 in Tris-HCl buffer with 1 μg/mL WGA-HRP and incubated at 37 °C for 15 min with shaking, and then washed five times with 50 mM Tris–HCl buffer at pH 7.5. Finally, 150 μL peroxidase substrate solutions (0.8 mM TMB, 2 mM H_2O_2, 50 mM Na_2HPO_4-Citric acid buffer at pH3.7) was added for lucifuge reaction time of 30 min at 25 °C. The reaction was stopped with 50 μL 2M H_2SO_4 and measured with Tecan Infinite 200 PRO Microplate reader at 450 nm. Draw the curve of concentration with (OD_0-B_0), and finally determine activity of the CHS.

[OD_0: Blank absorbance without inhibitor, substrate, and CHS.

B_0: Add CHS and substrate, without inhibition of HRP absorbance.]

4.2.3. Determination of inhibitors IC_{50} assay:

The stock solution of test compound was prepared by dissolving 10 mg of the test compound in 1 mL of DMSO solution. The stock solution was diluted to 300, 150, 75, 37.5, 18.75 μg/mL with 50mM Tris–HCl buffer at pH 7.5 as test solutions.

Plates stored at −20 °C were thawed at room temperature, emptied by pipettes and washed three times with 50 mM Tris–HCl buffer at pH 7.5. Reaction substrate (100 μL ,40 mM GlcNAc, 2mM UDP–GlcNAc, in 0.1M Tris-Maleic acid buffer at pH 6.8), 50μL test solution of compound and 50 μL diluted solution of CHS were added to appropriate wells. The positive control was added with 50 μL 0.1 M Tris-maleic acid buffer at pH 6.8 and 50 μL of CHS diluted solution. Blank control was added with 100 μL 0.1 M Tris- Maleic acid buffer at H 6.8. The plates were incubated at 37 °C for 1 h. The followed steps were the same as determination the activity of Chitin Synthase. Inhibition rate = $(B_o - B_n)/(B_0 - OD_{0)}$. The curve of log c and inhibition rate is plotted by IC_{50}.

B_0: Add enzyme and substrate, without inhibition of HRP absorbance.

B_n: Absorbance of HRP at a certain concentration

OD_0: Blank absorbance without inhibitor, substrate, and enzyme.

4.2.4. Antifungal activity assays [6]:

Antifungal activity was screened against four main pathogenic fungal species (*C. albicans* CMCC 76615, *A. fumigatus* GIMCC 3.19, *C. neoformans* ATCC 32719 and *A. flavus* ATCC 16870) in clinic. Fluconazole and polyoxin B used as standard antifungal drugs. DMSO was used as a solvent control. A spore suspension in sterile distilled water was prepared from 1-day old culture of the fungi growing on the media containing 1% peptone, 2% Glucose and solid

media as well as 15% agar. The final spore concentration was 1-5 $\times 10^3$ spore mL^{-1} All target compounds were dissolved in DMSO to prepare the stock solutions. The tests were made resulting in twelve wanted concentrations (0.25-512 mg/mL). These dilutions were incubated at 37 °C for 24 h. The MIC values of antifungal activity in mg/mL were summarized in Table 2.

4.2.5. The checkboard synergy analysis[26, 27]:

Two-dimensional broth microdilution checkboard assay between **6m** and **7** with fluconazole was carried out according to CLSI M27-A3 guidelines[28]. Each test was performed twice using 96-well plates. The plates were incubated for 48 hrs. at 35°C. It is important to say that the MIC values showed in Table. 3 were determined again in this investigation to allow fair comparison.

Using the obtained data, the FIC index for each combination was calculated. FIC index is calculated using the formula: FIC index = [(MIC_A in combination)/ MIC_A alone] + [(MIC_B in combination)/ MIC_B alone)]. Drug interactions were classified as synergistic if the FIC index was ≤0.5, indifferent if >0.5–4, and antagonistic if >4.

Declaration of interest

The authors report no declarations of interest.

Funding:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References:

ACCEPTED MANUSCRIPT

[1] E.M. Carmona, A.H. Limper, Overview of Treatment Approaches for Fungal Infections, Clin. Chest Med., (2017).
[2] J. Webster, R. Weber, Introduction to fungi, Cambridge University Press, 2007.
[3] K.M. Pianalto, J.A. Alspaugh, New Horizons in antifungal therapy, J. Fungi, 2 (2016) 26.
[4] M.K. Kathiravan, A.B. Salake, A.S. Chothe, P.B. Dudhe, R.P. Watode, M.S. Mukta, S. Gadhwe, The biology and chemistry of antifungal agents: a review, Bioorg. Med. Chem., 20 (2012) 5678-5698.
[5] S. Campoy, J.L. Adrio, Antifungals, Biochem. Pharmacol., 133 (2017) 86-96.
[6] Q. Ji, Z. Ge, Z. Ge, K. Chen, H. Wu, X. Liu, Y. Huang, L. Yuan, X. Yang, F. Liao, Synthesis and biological evaluation of novel phosphoramidate derivatives of coumarin as chitin synthase inhibitors and antifungal agents, Eur. J. Med. Chem., 108 (2016) 166-176.
[7] P. M Chaudhary, S. G Tupe, M. V Deshpande, Chitin synthase inhibitors as antifungal agents, Mini-Rev. Med. Chem., 13 (2013) 222-236.
[8] M.D. Lenardon, C.A. Munro, N.A. Gow, Chitin synthesis and fungal pathogenesis, Curr. Opin. Microbiol., 13 (2010) 416-423.
[9] H.C. Dorfmueller, A.T. Ferenbach, V.S. Borodkin, D.M. van Aalten, A structural and biochemical model of processive chitin synthesis, J. Biol. Chem., 289 (2014) 23020-23028.
[10] K.E. Jackson, P.K. Pogula, S.E. Patterson, Polyoxin and nikkomycin analogs: recent design and synthesis of novel peptidyl nucleosides, Heterocycl. Commun., 19 (2013) 375-386.
[11] S.K. Shahid, Newer patents in antimycotic therapy, Pat. Anal., 5 (2016) 115-134.
[12] L.F. Shubitz, H.T. Trinh, R.H. Perrill, C.M. Thompson, N.J. Hanan, J.N. Galgiani, D.E. Nix, Modeling nikkomycin Z dosing and pharmacology in murine pulmonary coccidioidomycosis preparatory to phase 2 clinical trials, The J. Infect. Dis., 209 (2014) 1949-1954.
[13] Q. Ji, D. Yang, X. Wang, C. Chen, Q. Deng, Z. Ge, L. Yuan, X. Yang, F. Liao, Design, synthesis and evaluation of novel quinazoline-2, 4-dione derivatives as chitin synthase inhibitors and antifungal agents, Bioorg. Med. Chem., 22 (2014) 3405-3413.
[14] Z. Ge, Q. Ji, C. Chen, Q. Liao, H. Wu, X. Liu, Y. Huang, L. Yuan, F. Liao, Synthesis and biological evaluation of novel 3-substituted amino-4-hydroxylcoumarin derivatives as chitin synthase inhibitors and antifungal agents, J. Enzyme Inhib. Med. Chem., 31 (2016) 219-228.
[15] A.R. Gholap, K.S. Toti, F. Shirazi, R. Kumari, M.K. Bhat, M.V. Deshpande, K.V. Srinivasan, Synthesis and evaluation of antifungal properties of a series of the novel 2-amino-5-oxo-4-phenyl-5, 6, 7, 8-tetrahydroquinoline-3-carbonitrile and its analogues, Bioorg. Med. Chem., 15 (2007) 6705-6715.

[16] J.M. Urbina, J.C. Cortés, A. Palma, S.N. López, S.A. Zacchino, R.D. Enriz, J.C. Ribas, V.V. Kouznetzov, Inhibitors of the fungal cell wall. Synthesis of 4-aryl-4-N-arylamine-1-butenes and related compounds with inhibitory activities on β (1–3) glucan and chitin synthases, Bioorg. Med. Chem., 8 (2000) 691-698.
[17] M. Sudoh, T. Yamazaki, K. Masubuchi, M. Taniguchi, N. Shimma, M. Arisawa, H. Yamada-Okabe, Identification of a Novel Inhibitor Specific to the Fungal Chitin Synthase Inhibition of chitin synthase 1 arrests the cell growth, but inhibition of chitin synthase 1 and 2 is lethal in the pathogenic fungus candida albicans, J. Biol. Chem., 275 (2000) 32901-32905.
[18] S. Ke, F. Liu, N. Wang, Q. Yang, X. Qian, 1, 3, 4-Oxadiazoline derivatives as novel potential inhibitors targeting chitin biosynthesis: Design, synthesis and biological evaluation, Bioorg. Med. Chem. lett., 19 (2009) 332-335.
[19] S.N. Lopez, M.V. Castelli, S.A. Zacchino, J.N. Domínguez, G. Lobo, J. Charris-Charris, J.C. Cortés, J.C. Ribas, C. Devia, A.M. Rodríguez, In vitro antifungal evaluation and structure–activity relationships of a new series of chalcone derivatives and synthetic analogues, with inhibitory properties against polymers of the fungal cell wall, Bioorg. Med. Chem., 9 (2001) 1999-2013.
[20] H.-B. Liu, H. Tang, D. Yang, Q. Deng, L.-J. Yuan, Q.-G. Ji, Synthesis and biological evaluation of novel N-acyl substituted quinolin-2 (1H)-one derivatives as potential antimicrobial agents, Bioorg. Med. Chem. lett., 22 (2012) 5845-5848.
[21] A.R. Yeager, N.S. Finney, The first direct evaluation of the two-active site mechanism for chitin synthase, J. Org. Chem., 69 (2004) 613-618.
[22] A.R. Yeager, N.S. Finney, Second-generation dimeric inhibitors of chitin synthase, Bioorg. Med. Chem., 12 (2004) 6451-6460.
[23] T. Lorand, B. Kocsis, Recent advances in antifungal agents, Mini-Rev. Med. Chem., 7 (2007) 900-911.
[24] K. Kral, T. Bieg, U. Nawrot, K. Włodarczyk, A. Lalik, P. Hahn, I. Wandzik, New monomeric and dimeric uridinyl derivatives as inhibitors of chitin synthase, Bioorg. Chem., 61 (2015) 13-20.
[25] H.A. Lucero, M.J. Kuranda, D.A. Bulik, A nonradioactive, high throughput assay for chitin synthase activity, Anal. Biochem., 305 (2002) 97-105.
[26] S.K. Shrestha, M.Y. Fosso, S. Garneau-Tsodikova, A combination approach to treating fungal infections, Sci. Rep., 5 (2015) 17070.
[27] S.K. Shrestha, M. Grilley, T. Anderson, C. Dhiman, J. Oblad, C.-W.T. Chang, K.N. Sorensen, J.Y. Takemoto, In vitro antifungal synergy between amphiphilic aminoglycoside K20 and azoles against Candida species and Cryptococcus neoformans, Med. Mycol., 53 (2015) 837-844.
[28] P. Wayne, Clinical and Laboratory Standards Institute: Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard, CLSI document M27-A3 and Supplement S, 3 (2008).

ACCEPTED MANUSCRIPT

Tables

Design, Synthesis and Biological Evaluation of Novel Quinazoline-2,4-diones Conjugated with Different Amino Acids as Potential Chitin Synthase Inhibitors

Nada A. Noureldin[a]*, Hend Kothayer[a*1], EL-Sayed M. Lashine[a], Mohamed M. Baraka[a], Yanrong Huang[b], Bing Li[b] and Qinggang Ji[b].

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

[b] *School of chemistry and chemical engineering, Southwest University, P.R.China*

ACCEPTED MANUSCRIPT

[1]Corresponding author. Tel. 00201224578259; E-mail: hendo1311@hotmail.com or hkelhamalawy@pharmacy.zu.edu.eg
*equal contribution

ACCEPTED MANUSCRIPT

COMP.	IP（%）	IC_{50}(mmol/L)	COMP.	IP（%）	IC_{50}(mmol/L)
6a	22.6	-	6i	43.1	-
6b	47.5	-	6j	50.5	-
6c	68.2	0.30	6k	43.8	-
6d	47.8	-	6l	61.5	0.30
6e	13.2	-	6m	75.5	0.18
6f	18.5	-	7	65.3	0.166
6g	52.8	-	Polyoxin B	63.9	0.17
6h	56.2				

Table. 1. The inhibition percent (IP) of compounds at concentration of 300μg/mL and IC_{50} values of some compounds.

Compound	***Aspergillus fumigates***	***Aspergillus flavus***	***Crytococcus neoformans***	***Candida albicans***
6a	>1.75	0.87	0.87	>1.75
6b	0.83	1.67	0.83	1.67
6c	0.80	1.60	0.80	1.60
6d	0.83	0.83	0.83	1.67
6e	0.80	1.60	0.80	>1.60
6f	0.76	>1.53	0.76	>1.53
6g	0.73	>1.47	>1.47	>1.47
6h	0.79	1.59	>1.59	>1.59
6i	1.40	>1.40	0.70	>1.40
6j	>1.34	>1.34	>1.34	>1.34
6k	>1.21	0.60	0.60	>1.21
6l	1.37	>1.37	0.68	>1.37
6m	0.77	1.54	0.77	>1.54
7	0.41	0.41	0.41	0.83
Fluconazole	0.05	0.10	0.10	0.20
Polyoxin B	0.12	0.12	0.06	0.12

Table. 2. The MIC value（mmol/L）of compounds 6a-m and 7 against fungi in vitro.

fungi	MIC(mmol/L)[a]							FIC Index[b] (6m)	FIC Index[b] (7)
	Alone			In combination					
	6m	**7**	fluconazole	**6m**	**7**	Fluconazole (with **6m**)	Fluconazole (with **7**)		
***Candida albicans* ATCC 76615**	0.773	0.417	0.104	0.386	0.417	0.013	0.013	0.625	1.125
***Aspergillus flavus* ATCC 16870**	0.773	0.417	0.104	0.193	0.209	0.007	0.026	0.3125	0.625
***Aspergillus fumigates* GIMCC 3.19**	1.545	0.834	0.209	1.545	0.209	0.013	0.052	1.0625	0.3125
Crytococcus neofonmans ATCC 32719	0.773	0.417	0.104	0.386	0.026	0.104	0.052	1.5	0.5625

Table 3. In vitro susceptibility of fungi to **6m**, **7** and fluconazole alone and in combination.

[a] All experiments were performed in duplicate. b Fractional inhibitory concentration index. Drug interactions were classified as synergistic if the FIC index was ≤0.5, indifferent if >0.5–4, and antagonistic if >4. From the activity data.

ACCEPTED MANUSCRIPT

Figures and scheme

Design, Synthesis and Biological Evaluation of Novel Quinazoline-2,4-diones Conjugated with Different Amino Acids as Potential Chitin Synthase Inhibitors

Nada A. Noureldin[a]*, Hend Kothayer[a*1], EL-Sayed M. Lashine[a], Mohamed M. Baraka[a], Yanrong Huang[b], Bing Li[b] and Qinggang Ji[b].

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

[b] *School of chemistry and chemical engineering, Southwest University, P.R.China*

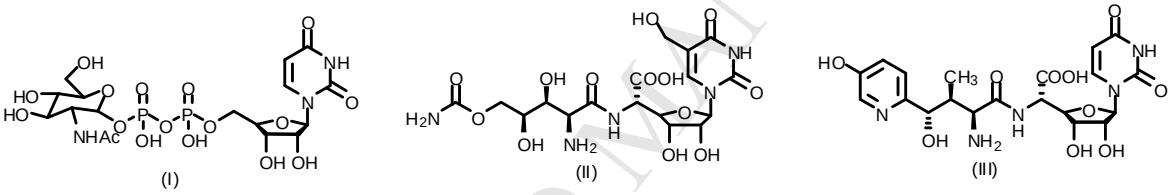


Figure. 1: (I) UDP-GlcNAc, (II) Polyoxin B, (III) Nikkomycin Z

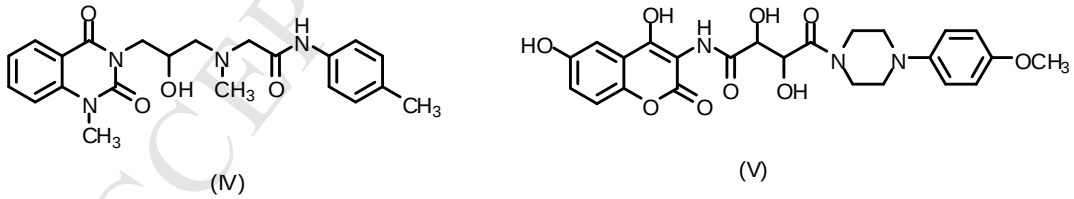


Figure. 2: Non-nucleoside organic compounds designed to roughly resemble UDP-GlcNAc.

[1]Corresponding author. Tel. 00201224578259; E-mail: hendo1311@hotmail.com or hkelhamalawy@pharmacy.zu.edu.eg
*equal contribution

ACCEPTED MANUSCRIPT

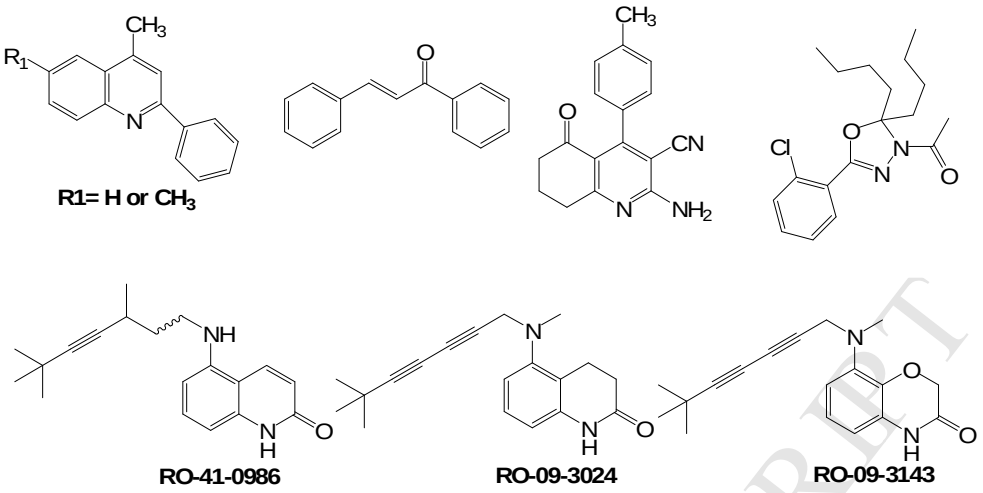


Figure. 3: Some examples on non-nucleoside small organic compounds with promising chitin synthase inhibitory activity.

ACCEPTED MANUSCRIPT

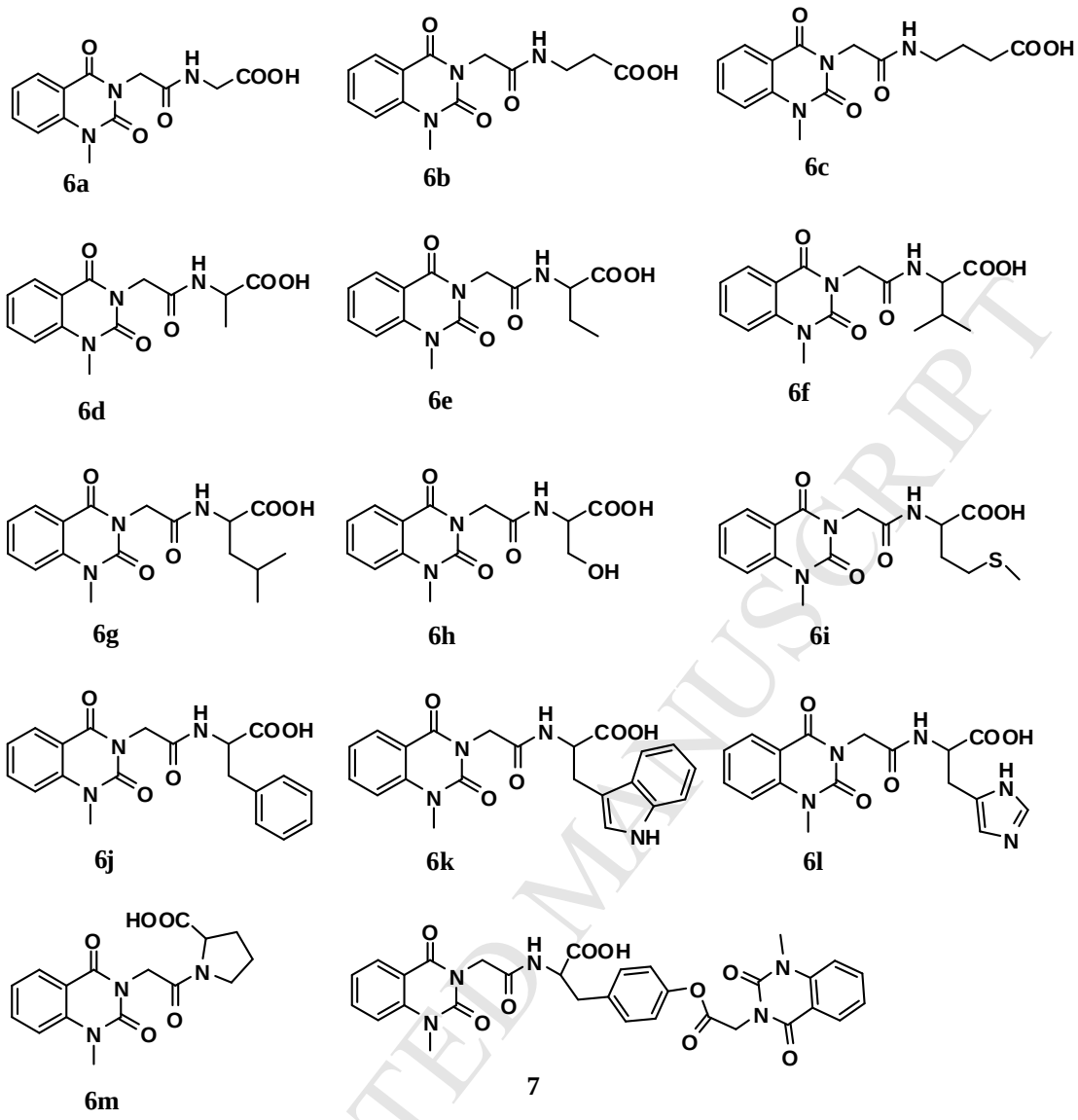


Figure.4: The diverse structures of the synthesized compounds (**6a-m** and **7**)

ACCEPTED MANUSCRIPT

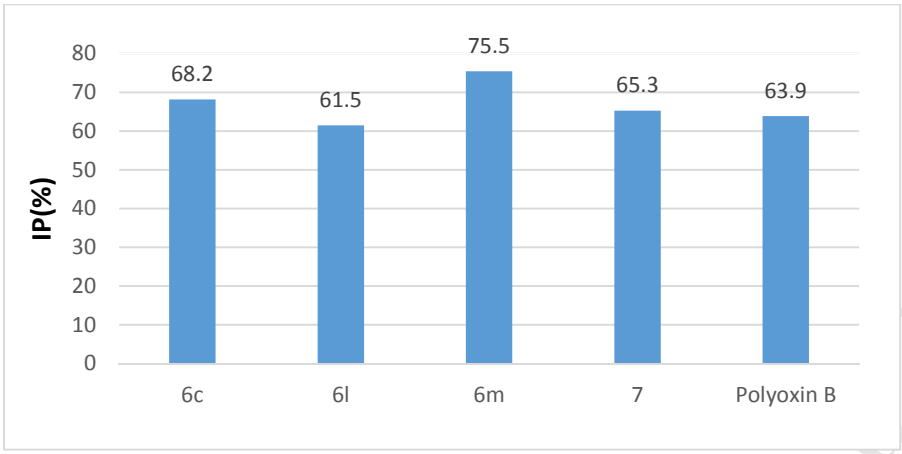


Fig. 5. The inhibition percent (IP) of compounds at concentration of 300μg/mL

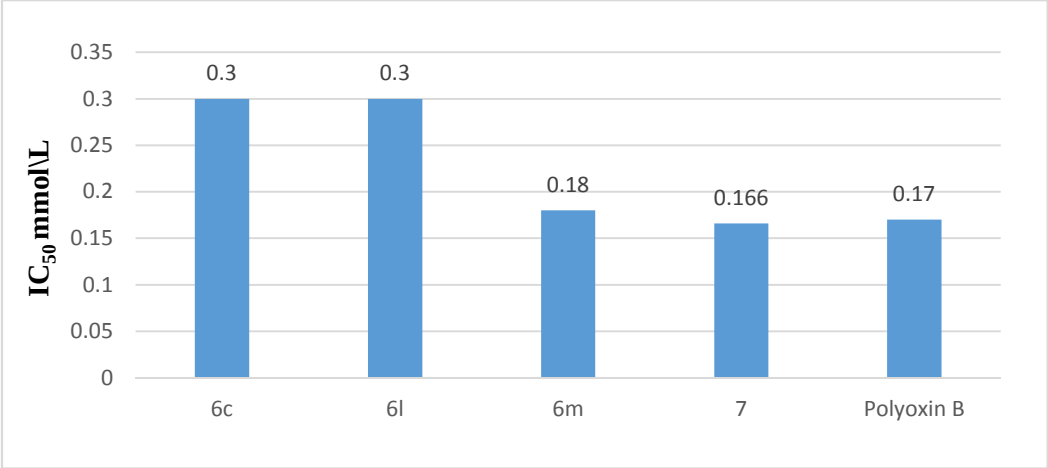
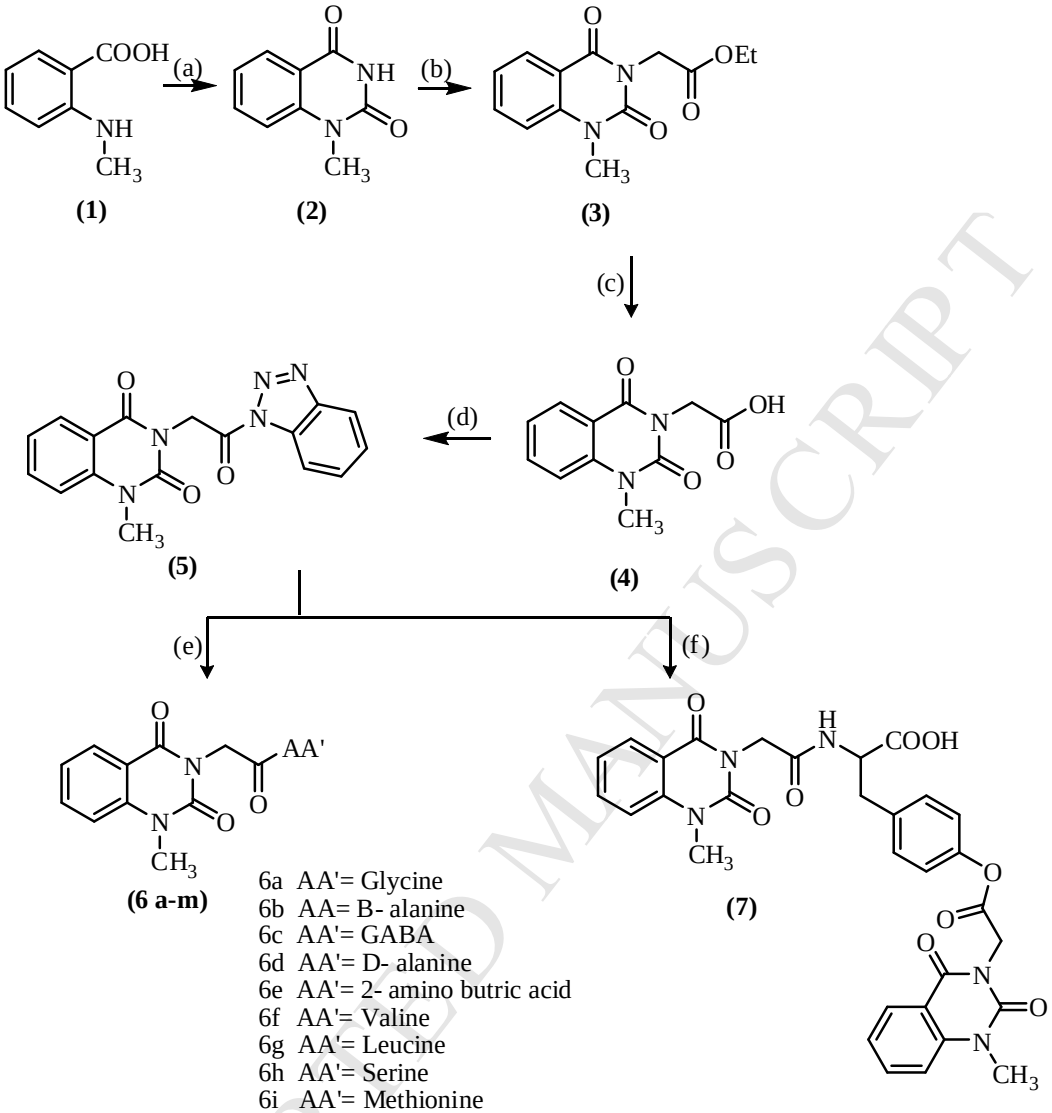


Fig. 6. The IC_{50} values of compounds whose IPs are more than 60%.



Scheme 1. Synthetic route of target compounds.Reagents and conditions: (a) NH_2CONH_2, 150°C, 12h; (b) $ClCH_2CO_2C_2H_5$, K_2CO_3, DMF, reflux, 12h; (c) NaOH, CH_3OH, reflux, 2h, then acidified with 1N HCl; (d) 1H-benzotriazole, CH_2Cl_2, $SOCl_2$, rt, 4.5 h; (e) appropriate amino acid, NEt_3, acetonitril/water (3:1), rt, 24 h; (f) Tyrosine, NEt_3, acetonitril/water (3:1), rt, 24h.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Highlights

Design, Synthesis and Biological Evaluation of Novel Quinazoline-2,4-diones Conjugated with Different Amino Acids as Potential Chitin Synthase Inhibitors

Nada A. Noureldin[a]*, Hend Kothayer[a*1], EL-Sayed M. Lashine[a], Mohamed M. Baraka[a], Yanrong Huang[b], Bing Li[b] and Qinggang Ji[b].

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

[b] *School of chemistry and chemical engineering, Southwest University, P.R.China*

- Series of quinazoline-2,4-diones **(6a-m)** and **(7)** have been synthesized.
- The new compounds were evaluated for their Chitin Synthase inhibitory activity.
- Compounds **6c, 6l, 6m** and **7** exhibited the most promising enzyme inhibitory activity.
- Compound **7** with its dimeric structure showed the highest potency among others.

[1]Corresponding author. Tel. 00201224578259; E-mail: hendo1311@hotmail.com or hkelhamalawy@pharmacy.zu.edu.eg
*equal contribution