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Identification of novel quinazolin-4(3*H*)-ones as inhibitors of thermolysin, the prototype of the M4 family of proteinases

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ABSTRACT

A combinatorial series of novel quinazolin-4(3*H*)-ones were synthesised and their structures were established based on spectroscopic data (IR, NMR, EI-MS, and FAB-MS). The compounds were tested for inhibition of the zinc metalloproteinase thermolysin (TLN) utilizing a chemical array-based approach. Some of the compounds were found to inhibit TLN, with IC_{50} values ranging from 0.0115 μM (compound **3**) to 122,637 μM (compound **29**). Compound **3** [3-phenyl-2-(trifluoromethyl) quinazolin-4(3*H*)-one] (IC_{50} = 0.0115 μM) and compound **35** [3-(isopropylideneamino)-2,2-dimethyl-2,3-dihydroquinazolin-4 (1*H*)-one] (IC_{50} = 0.2477 μM) were found to be the most potent inhibitors.

1. Introduction

Thermolysin (TLN, EC 3.4.24.27) is a zinc-containing eubacterial endoproteinase from *Bacillus thermoproteolyticus*, known as the prototype of the M4 family of proteinases. The 3D structure of TLN contains a single catalytic Zn^{2+} ion essential for hydrolytic activity, and four Ca^{2+} ions required for thermostability.[1,2] The enzyme catalyzes hydrolysis of peptide bonds containing hydrophobic amino acid residues.[3–6] Enzymes of the M4 family are important for suppressing or avoiding the innate immune system of infected host during pathogenesis,[7–11] and therapeutic inhibition of several M4 enzymes is believed to be a novel strategy in development of a new generation of antibacterial drugs.[12,13]

X-ray crystallographic studies have shown that TLN contains a HExxH+E motif that forms a part of the zinc-binding site.[14] Zn-metalloproteinases that contain the HExxH+E motif are often named thermolysin-like proteinases (TLPs). TLPs consist of about 750 amino acids, with a signal- and pro-sequence of 200–250 residues, a catalytic domain of about 300 residues, and a C-terminal domain of about 100 amino acids. Angiotensin I-converting enzyme (ACE, EC 3.4.15.1) and neprilysin (NEP, EC 3.4.24.11) are therapeutically important TLPs involved in controlling the blood pressure.[15,16] ACE and TLN share structural and catalytic similarities that previously have been discussed.[17–19] The membrane-bound metalloproteinase NEP has a catalytic unit similar to that of TLN and ACE; and the catalytic properties resemble those of TLN.[20,21] Due to functional and structural conservations, TLN inhibitors are often inhibitors of ACE and NEP and other structurally related Zn-metalloproteinases. ACE inhibitors are first line therapy for hypertension, heart failure, myocardial infarction, and diabetic nephropathy.[22] Based on the structural and functional similarities, TLN is considered a perfect model protein for studying TLPs.

Quinazolin-4(3*H*)-one is a heterocyclic alkaloid ring system frequently encountered in medicinal chemistry. These alkaloids have attracted much focus by synthetic and medicinal chemists and several reports are available describing synthesis and activity. Quinazolin-4(3*H*)-one structure analogous have been found to have different biological properties including anticonvulsant, sedative, tranquilizer, analgesic, antimicrobial, anesthetic, anticancer, antiviral, antihypertensive, anti-inflammatory, diuretic, and muscle relaxant.[23–31] In the present study, we have synthesised 2,3-disubstituted quinazolin-4(3*H*)-one derivatives and studied their TLN inhibition using in vitro binding assays.

Abbreviations: ACE, angiotensin I-converting enzyme; EC, enzyme commission; FaGLa, *N*-[3-(2-furyl) acryloyl] glycyl-L-leucinamide; NEP, neprilysin; PDB, protein databank; TLC, thin layer chromatography; TLP, thermolysin-like proteinase; TLN, thermolysin.

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2. Results and discussion

2.1. Chemistry

Three different methods were used for the synthesis of 2,3-disubstituted quinazolin-4(3*H*)-ones (Scheme 1). The synthesis of 2-aryl 3-amino quinazolin-4(3*H*)-ones was accomplished as recently reported, via a benzoxazinone intermediate,[32] while 2-aryl 3-alkyl/aryl quinazolin-4(3*H*)-ones were synthesized by refluxing 2-arylbenzoxazinones with selected aromatic/aliphatic amines (Scheme 1).[30,33,34] 3-(2-Hydroxyethyl) quinazolin-4(3*H*)-one (**15**) and 3-(2-hydroxyethyl)-2-methylquinazolin-4(3*H*)-one (**16**) were synthesised using PTSA catalyzed three component one-pot synthesis.[35] The synthesis of 2-triflouro-3-amino/3-phenyl quinazolin-4(3*H*)-ones (**2** and **3**) (Scheme 1) was accomplished using microwave irradiations. A new approach was used for the synthesis of methyl 2-[(trifluoroacetyl) amino] benzoate (**1**) by reacting trifluoroacetic acid with methylanthranilate under microwave irradiations (Scheme 1). The structurally novel quinazolin-4(3*H*)-one derivative (**17**) was synthesised by reacting 2-phenyl benzoxazinones with propylenediamine in dry pyridine (Scheme 1). In an effort to synthesize structurally more diverse quinazolin-4(3*H*)-one derivatives in search for potent molecules, 2-aryl/alky 3-amino quinazolin-4(3*H*)-ones were used as precursors.

In the first attempt a series of amides (**18–22**) were synthesised (Scheme 2) using simple reaction conditions, that is, by refluxing chloroacetylchloride and 2-aryl/alky 3-amino quinazolin-4(3*H*)-ones in dry benzene[36,37] or by stirring 2-aryl/alky 3-amino quinazolin-4(3*H*)-ones and acid halide in dry pyridine at room temperature for appropriate time. Quinazolin-4(3*H*)-one Schiff bases have been reported to have several biological activities.[36,38–40] Therefore, we attempted to prepare a series of quinazolin-4(3*H*)-one Schiff bases. There are limited reports available about synthesis of quinazolin-4(3*H*)-one Schiff bases, but improved yield in shorter reaction time was achieved after necessary modification of reported methods. In a typical reaction run, selected aromatic aldehydes were stirred in dry ethanol for 15–30 min. using 2–3 drops H_2SO_4 as catalyst followed by addition of 2-aryl/alky 3-amino quinazolin-4(3*H*)-ones and refluxing further (Scheme 2).

Synthetic acetamidine compounds are reported to affect many biological processes,[41–43] but no report about quinazolin-4(3*H*)-one based on acetamidine is available. A novel quinazolin-4(3*H*)-one based acetamidine (**30**) was therefore synthesised (Scheme 2).

The structure–activity relationship (SAR) of the compounds indicated that position 3 is important for activity, and 2-alkyl/aryl quinazolin-4(3*H*)-ones (**31–34**) (Scheme 3) were therefore synthesised via oxidative deamination of 2-aryl/alky 3-amino quinazolin-4(3*H*)-ones using a recently reported method.[44]

Synthetic routes for compounds **35–37** are shown in Scheme 3 and 4. The structure of the synthesised compounds was determined on the bases of spectroscopic data, including, IR, NMR, EI-MS, and FAB-MS as reported in the experimental section.

2.2. TLN inhibition

IC_{50} values for the compounds were determined using *N*-[3-(2-furyl)acryloyl]glycyl-L-leucinamide (FaGLa) as TLN substrate. The IC_{50} values are given in Table 1. The in vitro results indicated that 12 out of 37 compounds were inhibitors, while 21 compounds showed very low inhibition, and the IC_{50} values could not be detected. Four compounds were completely inactive. The IC_{50} values of compound **3** (IC_{50} = 0.0115 μM), compound **31** (IC_{50} = 1.25 μM) and compound **35** (IC_{50} = 0.2477 μM) were more favorable than for the other compounds. K_i values of active compounds were derived from the Cheng–Prusoff relationship based on IC_{50} values.[45–47] Enzymes kinetic values were based on initial velocities determined for ⩽10% of the reaction. The molecular structures of the compounds are shown in Schemes 1–4.

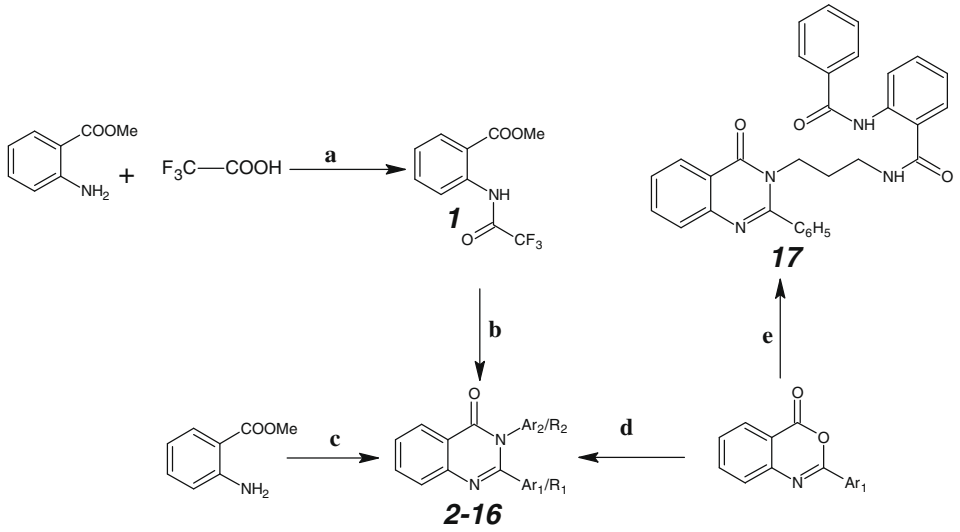
Replacement of the amino group in position 3 of compound **2** with a phenyl ring giving compound **3** (Fig. 1) caused an affinity increase of more than 3000 times. Further, the affinity of compound **29** is lower than that of compounds **4**, **23**, and **25**. Based on these observations it seems like an aromatic substituent in position 3 is favorable for strong TLN affinity of the quainzolin-4(3*H*)-ones (Table 1). Interestingly the position of the substituent on the aromatic group in position 3 also seems important. When the chloride in *para* position of the aromatic group (compound **4**) is changed to *meta* (compound **25**) the affinity drops. However, when the chloride in *para* position (compound **4**) is replaced with a dimethylamino group (compound **23**) the affinity is almost similar (Table 1).

Compounds with quite small substituent in position 2 (compounds **2**, **3**, **31**, and **35**) seem to have higher affinity than the compounds with a larger group. A trifluoromethyl group seems more favorable in position 2 than aromatic bulky substituents since compound **2** binds stronger than **8** and **13**. However, it seems like a strong electronegative group in position 2 is favorable since analogous with a methyl group in position 2 binds with lower affinity (compound **24**), or not at all (compounds **16**, **19**, **22**). The contribution of trifluoromethyl group in position 2 may be position specific since compound **1** (Scheme 1) only gave low TLN inhibition compared with compound **2** and **3**.

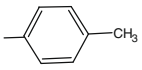
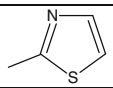
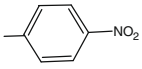
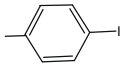
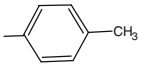
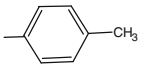
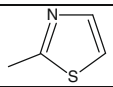
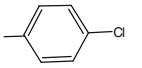
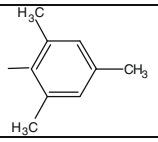
Based on the experimental results it seems like compounds with an aromatic electron rich group in position 3 and a strong electronegative group in position 2 (trifluoromethyl group) of the quainzolin-4(3*H*)-one ring system seems favorable for strong TLN binding and inhibition by compounds of the present series.

The fluorinated compounds display a number of interesting physiochemical and biological properties which make them attractive in drug discovery and in carrying, targeting and delivering devices.[48,49] Strong electron withdrawing properties of the fluorine may effects acidity and pK_a values of neighboring functional groups and amino acids in the target enzyme, and may thereby effects binding affinity. The trifluoromethyl group of compound **2** and **3** can lower the basicity of the quinazolin ring system and affect the property of the substituent in position 3.[50] A search in the PDB showed that 38 complexes of fluorine or trifluoromethyl substituted compounds in complex with enzymes classified as EC3 enzymes were known. The 38 complexes showed that the fluorine group populated either outside binding pocket, or was mainly surrounded by hydrophobic amino acids. This may indicate that the contribution of fluorine on neighboring functional groups is more important for binding affinity than a direct interaction between the fluorine group and amino acids in the enzyme.

The experimental studies indicated that a trifluoromethyl group in position 2 is more favorable than a phenyl ring or a *para* substituted phenyl ring for affinity. Molecular docking of compound **3**, according to previously described methods,[51] indicated that the trifluoromethyl group in position 2 of compound **3** interacted with TLN in the region of Asn112 (S1′-subsite) and Phe114 (S1-subsite) as shown in Figure 1B. However, the packing around the trifluoromethyl group was not as tight as seen for other parts of the compound, and the trifluoromethyl group was accessible from the surface. Docking of compound **3** may therefore also indicate that the main contribution of the trifluoromethyl group to favorable TLN affinities may be that the strong electron withdrawing properties of fluorine may affect the physiochemical properties of the neighboring quinazolin ring system and the substituent in position 3. The experimental binding studies indicated that aromatic

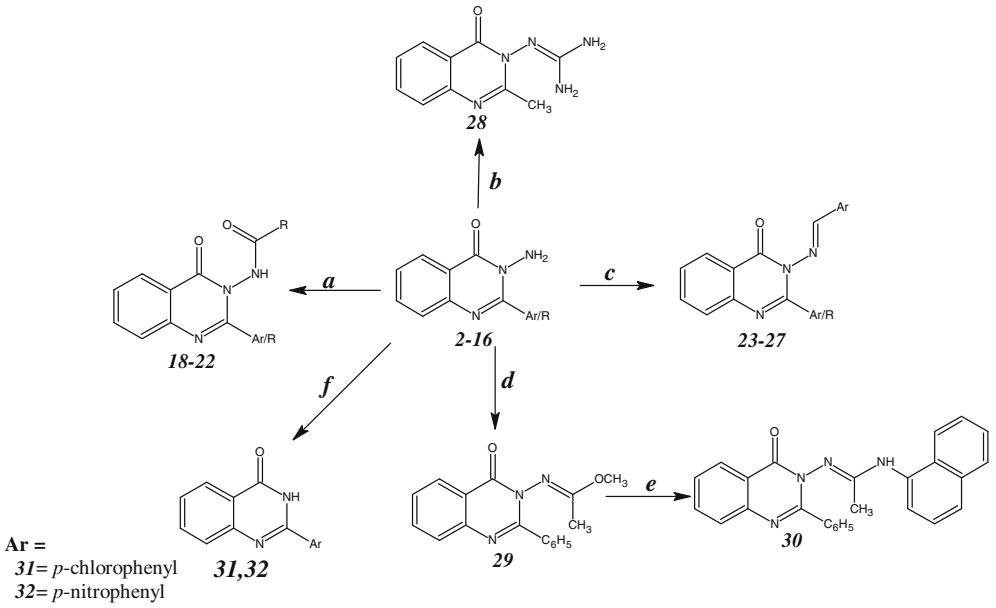


Compounds (**2-16**)

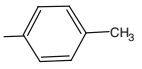
Compound	Ar_1/R_1	Ar_2/R_2
2	CF_3	NH_2
3	CF_3	
4	CH_3	NH_2
5	$NHNH_2$	NH_2
6		NH_2
7		
8		NH_2
9		
10		$-CH_2CH_2NH_2$
11		$-CH_2CH_2CH_2NH_2$
12		
13		NH_2
14		
15	H	$-CH_2CH_2OH$
16	CH_3	$-CH_2CH_2OH$

Scheme 1. Various routes for synthesis of compounds **2**-**17**. Reagents and conditions: (a) MW 1 min; (b) and (c) hydrazine hydrate, ethanol, reflux, 2 h; (d) hydrazine hydrate, pyridine or benzene, reflux, 6–8 h; (e) $NH_2CH_2CH_2CH_2NH_2$, reflux, dry benzene, 2 h.

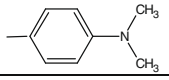
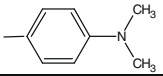
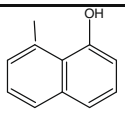
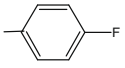
substituents in positions 3 increased the binding affinity (Table 1). Docking of compound **3**, indicated that the phenyl ring in position 3 interacted with Asn111, Asn112, and His231 in the region of S1′ and S2′ subsites. The oxygen atom connected to the quinazolin ring system is coordinating the catalytic zinc at the active site of TLN (Fig. 1).



Compounds (***18-22***)

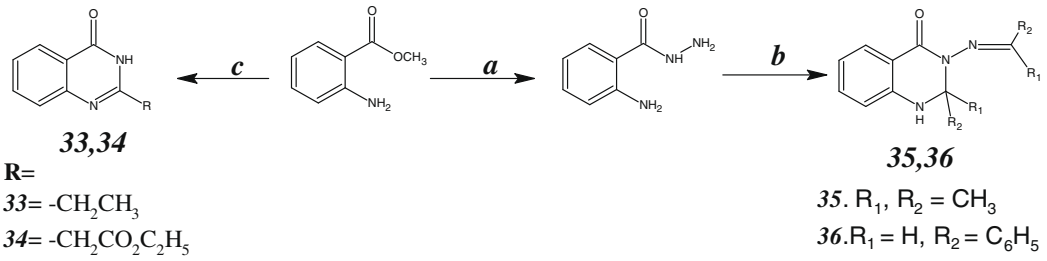
Compound	Ar/R	R
18		CH_3
19	CH_3	
20	CH_3	CH_3
21	CH_3	
22	CH_3	

Compounds (***23-27***)

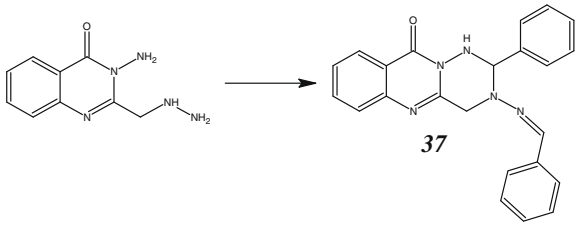
Compound	Ar/R	Ar
23		
24	CH_3	
25		
26		
27		

Scheme 2. Synthetic scheme of compounds **18**-**30**. Reagents and conditions: (a) acid halid, pyridine or benzene (dry), stir, 2–5 h; (b) urea, ethanol(dry), 5 h; (c) (i) ethanol dry, H_2SO_4, reflux; (ii) 5–10%, $NaHCO_3$; (d) triethylorthoacetate, stir; (e) acetic acid, reflux, 6 h; (f) $KMnO_4$, water, reflux.

In a previous study based on virtual screening of the US National Cancer Institute compound database, we identified 12 compounds as TLN inhibitors.[51] In general the quinazolin derivatives tested in the present study had higher TLN affinity than most of the compounds in the previous study. Docking of the 12 compounds indentified as TLN inhibitors in the present study (Table 1) showed that they all coordinated the catalytic zinc, as show for compound **3** in Figure 1. However, docking of the binders in our



Scheme 3. Synthetic scheme of compounds **31-36.** Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, ethanol, reflux, 2 h; (b) aldehyde/ketone, silica gel, stir; (c) alkylcyanide, dioxane (dry), HCl (dry), stir.



Scheme 4. Synthetic scheme of compound **37.** Reagents and conditions: benzaldehyde, ethanol (dry), reflux, 2 h.

Table 1
TLN inhibitory activities (IC_{50} and K_i values) of 2,3-disubstituted quainzolin-4(3*H*)-ones. The K_i values were determined from IC_{50} values using the Cheng–Prusoff relationship

Compd	IC_{50} values (μM)	K_i (μM)	Compd	IC_{50} values (μM)	K_i (μM)
1	LA[a]	—	**20**	LA[a]	—
2	37.86	37.9	**21**	LA[a]	—
3	0.0115	0.0115	**22**	LA[a]	—
4	54.89	54.9	**23**	42.03	42.00
5	LA[a]	—	**24**	1832	1830
6	Inactive[b]	—	**25**	4002	4000
7	76.85	59.3	**26**	LA[a]	—
8	3118	3120	**27**	LA[a]	—
9	LA[a]	—	**28**	LA[a]	—
10	LA[a]	—	**29**	122,637	106,000
11	LA[a]	—	**30**	LA[a]	—
12	LA[a]	—	**31**	1.25	1.25
13	12,743	10,100	**32**	LA[a]	—
14	Inactive[b]	—	**33**	LA[a]	—
15	LA[a]	—	**34**	LA[a]	—
16	Inactive[b]	—	**35**	0.2477	0.243
17	LA[a]	—	**36**	LA[a]	—
18	LA[a]	—	**37**	Inactive [b]	—
19	LA[a]	—			

Notes: LA, low activity.
[a] Due to the low activity the IC_{50} values were not possible to calculate.
[b] Completely inactive.

previous study indicated that some of them did not interact directly with the zinc.[51] Direct zinc coordination is believed to increase the TLN affinity, and that may be the reason for the higher affinity of the best binders in the present study compared with those of the previous study.

3. Conclusions

Our study indicated that a trifluoromethyl group was favoured in position 2, while aromatic substituents were favoured in position 3 of the present series of quinazolin-4(3*H*)-ones. Compounds **3** [3-phenyl-2-(trifluoromethyl) quinazolin-4(3*H*)-one] (IC_{50} = 1.1461 × 10^{-2} μM) and **35** [3-(isopropylideneamino)-2,2-dimethyl-2,3-dihydroquinazolin-4(1*H*)-one] (IC_{50} = 2.4774 × 10^{-1} μM) were found to be the most potent TLN inhibitors. Docking of compound **3** into the active site of TLN indicated that the trifluoromethyl group in position 2 interacted in the region of Asn112 (S1′-subsite) and Phe114 (S1-subsite), while the phenyl ring in position 3 interacted Asn111, Asn112, and His231 in the S1′ and S2′ subsites. The present TLN inhibitors may function as 'lead' molecules for designing more potent TLN inhibitors with putative clinical application.

4. Materials and methods

4.1. Compound synthesis

Before synthesis all starting materials were purified using standard methods. TLC, performed on precoated Silica (E-Merck, Germany) plates; column chromatography (silica column) in addition to re-crystallization was used for purification. Solvents were purified and dried according to their standard methods. Melting points were measured in open capillaries using Gallenkamp melting point apparatus. IR (KBr) spectra were recorded on Bio-Radio-Wind IR, ^{1}H NMRs were recorded on Bruker 400 MHz, while FAB-MS and EI-MS were recorded on a Mat-312 instrument.

4.1.1. Methyl 2-[(trifluoroacetyl) amino] benzoate (1)

A mixture of methyl anthranilate (1.0 g, 0.00653 mol) and triflouroacetic acid in a capped tube was put into a Teflon cylinder container and microwave irradiated at three stages (20 s each) for 60 s. In order to precipitate the product, the mixture was cooled to room temperature and ice-cold water was added. The resulting mixture was then filtered, washed with cold water, dried and re-crystallized from ethanol. Yield: 97%; mp 157 °C; IR (KBr) ν_{max} cm^{-1}: 3234 (N–H), 3011 (C–H), 1675 (C=O), 1567 (C=O), 1542 (C=C), 1296 (C–N, C=N), 1233 (C–C): EI-MS *m*/*z*: 247 (M^+, 51), 150 (60), 119 (98), 105 (100), 92 (44), 76 (5), 65 (20), 51 (3): ^{1}H NMR (300 MHz, $CDCl_3$), δ 11.9 (s, b, 1H), 8.7 (d, 1H, *J* = 8), 8.22 (d, 1H, *J* = 8), 7.16 (t, 2H, *J* = 8), 3.94 (s, 3H, CH_3).

4.1.2. 3-Amino-2-(trifluoromethyl) quinazolin-4(3*H*)-one (2)

Methyl 2-[(trifluoroacetyl) amino] benzoate (2 g, 0.008 mol) and hydrazine hydrate 80% (10 ml) in absolute ethanol (10 ml) was reflux for 2 h after completion of the reaction as indicated by TLC. The reaction mixture was allowed to cool to room temperature and then poured into ice-cold water. The product precipitated on stirring was filtered, washed with distilled water, dried and re-crystallized from ethanol. Yield: 94%, mp 134 °C: IR (KBr) ν_{max} cm^{-1}: 3208 (N–H), 1665 (C=O), 1601 (C=N), 1530 (C=C), 1251 (C–N, C–O), 1188 (C–C); EI-MS *m*/*z*: 229 (100), 124 (3), 200 (53), 180 (13), 130 (26), 120 (6), 90 (5), 77 (3). ^{1}H NMR (300 MHz, $CDCl_3$), δ 8.32 (d, 1H, *J* = 9), 7.85 (t, 2H, *J* = 8), 7.65 (m, 1H), 4.89 (s, b, 2H).

4.1.3. 3-Phenyl-2-(trifluoromethyl) quinazolin-4(3*H*)-one (3)

A mixture of methyl 2-[(trifluoroacetyl) amino] benzoate (1 g, 0.004048 mol) and freshly distilled aniline (0.5 g, 0.005376 mol) was refluxed in absolute ethanol (10 ml) for 3 h. After completion

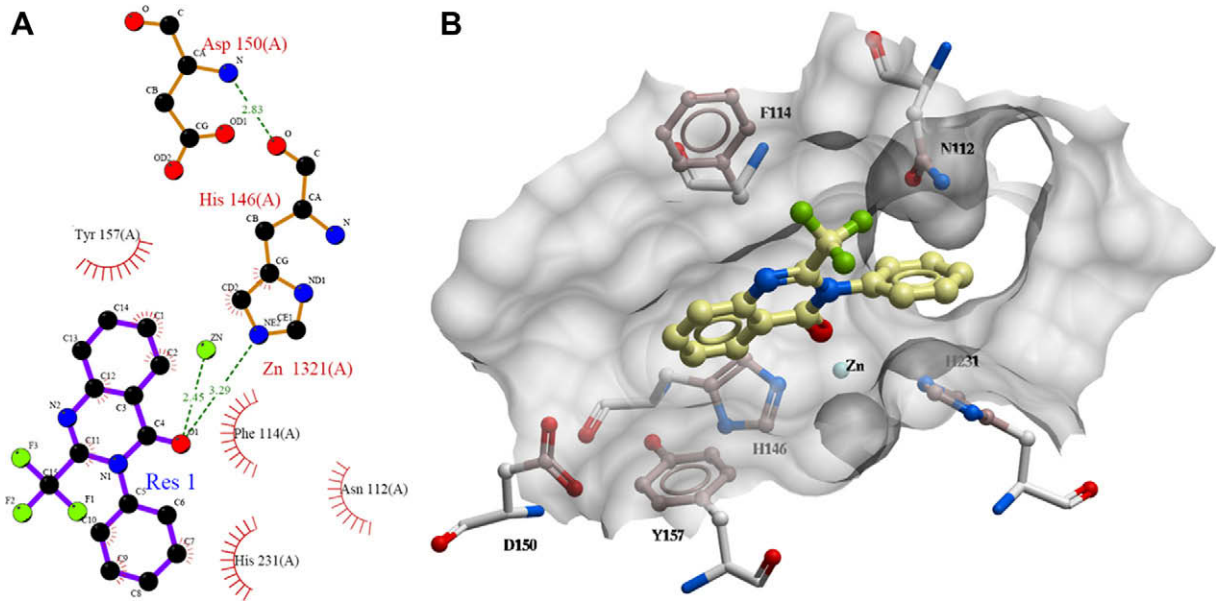


Figure 1. (A) Schematic illustration of the interactions of compound **3** at the active site of TLN as indentified by LigPlot. (B) Corresponding 3D view of compound **3** at the active site of TLN. The binding pocket is in grey transparent mode.

of the reaction, ethanol was evaporated under reduced pressure and the remaining mixture was poured into ice-cold water. The solid precipitated was filtered, washed with water and re-crystallized from ethanol. Yield: 91%, mp 135 °C; EI-MS *m*/*z*: 290 (M^+,61), 221 (31), 213 (49), 200 (31), 196 (4), 172 (27), 145 (100), 132 (12), 120 (19), 105 (81), 77 (8), 76 (11), 65 (7), 54 (5): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, 1H, J = 7.7), 8.01 (d, 1H, J = 8), 7.82 (d, 2H, J = 8), 7.71 (t, 1H, J = 8.1), 7.61 (t, 1H, J = 8), 7.53 (t, 1H, J = 8), 7.31 (m, 2H).

4.1.4. 3-Amino-2-methylquinazolin-4(3*H*)-one (4)

Methyl *N*-acetyl anthranilate (2 g., 0.01 mol) and hydrazine hydrate 80% (10 ml) were boiled under reflux for 2 h. After completion of the reaction (as indicated by TLC), the reaction mixture was cooled to room temperature and poured into ice-cold water. The product precipitating after stirring was filtered, washed with saturated $NaHCO_3$, and then with distilled water, dried and re-crystallized from water to give the target compound. Yield: 91%, mp 143 °C; IR (KBr) ν_{max} cm^{-1}: 3297 (NH), 2954 (C–H), 1721 (C=O), 1590 (C=N), 1563 (C=C), 1225 (C–O,C–N), 1193 (C–C); EI-MS *m*/*z*: 175 (M^+, 100), 145 (82), 120 (5), 117 (12), 90 (11), 76 (25), 65 (3), 51 (15). ^{1}H NMR (300 MHz, $CDCl_3$), δ 8.08 (d, 1H, J = 8), 7.76 (t, 1H, J = 10), 7.58 (d, 1H, J = 10), 7.46 (t, 1H, J = 10), 5.79 (s, 2H), 2.56 (s, 3H).

4.1.5. 3-Amino-2-(hydrazinomethyl) quinazolin-4(3*H*)-one (5)

Methyl 2-[(chloroacetyl) amino] benzoate (1 g, 0.0044 mol), hydrazine hydrate 80% (5 ml) in ethanol (10 ml) was refluxed for 2 h. Yield: 79%, mp 136–138 °C: IR (KBr) ν_{max} cm^{-1}: 3525 (N–H), 3010 (N–H), 1645 (C=O), 1532 (C=N), 1471 (C=C), 1251 (C–N, C–O), 1196 (C–C); FAB-MS (−ve, +ve); *m*/*z*: 204, 206. EI-MS *m*/*z*: 205 (3), 175 (68), 165 (6), 149 (19), 123 (13), 109 (25), 97 (43), 85 (68), 83 (100), 71 (54), 57 (85), 52 (52). ^{1}H NMR (300 MHz, $CDCl_3$), δ 8.26 (d, 1H, J = 9), 7.73 (t, 1H, J = 9), 7.63 (d, 1H, J = 9), 7.48 (t, 1H, J = 9), 4.8 (b, s, 5H, NH), 3.47 (s, 2H, CH_2).

4.1.6. 3-Amino-2-phenylquinazolin-4(3*H*)-one (6)

A mixture of 2-phenyl-4*H*-3,1-benzoxazine-4-one (2 g, 0.00896 mol) and hydrazine mono hydrate 80% (5 ml) in dry benzene (15 ml) was refluxed for 2 h. After completion of the reaction as indicated by TLC, the product precipitating upon cooling was further digested by dilute HCl solution for 1 h, and then cooled to room temperature. The precipitate formed was filtered, washed with water and re-crystallized from ethanol. Yield: 86%, mp: 236–238 °C; IR (KBr) ν_{max}: 3207 (N–H), 1768 (C=O), 1602 (C=N), 1484 (C=C), 1245 (C–O, C–C); EI-MS *m*/*z*: 237 (100), 236 (34), 222 (26), 221(12), 208 (59), 180 (18), 179 (7), 152 (7), 119 (37), 105 (10), 103 (8), 77 (28), 63 (5), 50 (14). ^{1}H NMR (300 MHz, $CDCl_3$), δ 8.35 (d, 1H, J = 8), 7.82 (m, 4H), 7.56 (m, 4H), 4.43 (s, 2H).

4.1.7. 2-(4-Methylphenyl)-3-(1,3-thiazol-2-yl) quinazolin-4(3*H*)-one (7)

2-(4-Methylphenyl)-4*H*-3,1-benzoxazin-4-one (0.05 g, 0.000 21097 mol) in dry pyridine (10 ml) was refluxed for 10 h. After completion of the reaction, the reaction mixture was poured in ice-cold water. The product precipitated was filter, dried and re-crystallized from ethanol. Yield: 78.3%, mp 212–214 °C; IR (KBr) ν_{max}: 1654 (C=O), 1591 (C=N), 1520 (C=C), 1301 (C–H, bend), 1245 (C–O), 1234 (C–C), 834, 756, 681 (C–H, arom. bend): EI-MS *m*/*z*: 319 (M^+, 2), 318 (12), 239 (43), 238 (100), 193 (7.5), 146 (19), 120 (18), 119 (100), 92 (6), 91 (41), 65 (10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.96 (d, 1H, J = 9), 8.29 (d, 1H, J = 9), 8.17 (t, 1H, J = 6), 7.94 (d, 2H, J = 9), 7.68 (m, 1H), 7.53 (s, 1H), 7.33–7.223 (m, 3H), 7.12 (d, 1H, J = 3), 6.8 (d, 1H, J = 3), 2.45 (s, 3H).

4.1.8. 3-Amino-2-(4-nitrophenyl)quinazolin-4(3*H*)-one (8)

2-(4-Nitrophenyl)-4*H*-3,1-benzoxazin-4-one (2 g), and hydrazine hydrate 80% (5 ml) in 15 ml benzene was refluxed for 5 h. The product was re-crystallized from ethanol. Yield: 91%, mp 202 °C: IR (KBr) ν_{max}: 3207 (NH), 3040 (CH), 1635 (C=O), 1529 (C=N), 1345 (C–H, bend), 1296 (C–O), 1233 (C=C), 840 (C–H, arom. bend), 709 (C–H, bend), 671 (C–H, bend, arom.): EI-MS *m*/*z*: 282 (M^+, 81), 180 (30), 253 (40), 236 (44), 207 (43), 179 (19), 177 (27), 105 (10), 92 (10), 90 (26), 76 (100), 65 (13), 51 (19). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.34 (d, 3H, J = 9), 8.02 (d, 2H, J = 9), 7.81 (s, 2H), 7.59 (m, 1H), 4.89 (b, s, 2H, N–H).

4.1.9. 3-(4-Iodophenyl)-2-phenylquinazolin-4(3*H*)-one (9)

This compound was prepared from 2-phenyl-4(*H*)-3,1-benzoxazine-4-one **2** (1 g, 0.00448 mol) and 4-iodoaniline (1 g, 0.00448 mol) in 10 ml dry benzene as described for compound **35**.

Yield: 88%, mp 231–235 °C; IR (KBr) ν_{max} cm^{-1}: 1664 (C=O), 1603 (C=N), 1534 (C=C, conj.), 1257 (C–C), 565 (C–I): EI-MS *m*/*z*: 424 (12), 348 (3), 297 (3), 222 (96), 209 (10), 181 (5), 146 (11), 120 (11), 105 (100), 92 (14), 77 (24), 65 (5), 51 (7); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.4 (d, 1H, *J* = 7.8), 7.98 (d, 2H, *J* = 15), 7.85–7.81 (t, 1H, *J* = 8), 7.6–7.54 (m, 4H), 7.5–7.45 (m, 3H), 7.3–7.28 (d, 2H, *J* = 11.5).

4.1.10. 3-(3-Aminoethyl)-2-(4-methylphenyl) quinazolin-4(3*H*)-one (10)

A mixture of 2-(4-methylphenyl)-4*H*-3,1-benzoxazin-4-one (1.7 g, 0.007 mol) and excess of 1,3-diaminoethaneane (0.967 ml, 0.0143 mol) in dry toluene (15 ml) was refluxed with continuous stirring for 6 h. After completion of the reaction (indicated by TLC), the reaction mixture was allowed to cool to room temperature and then poured into water. The product precipitating was filtered, washed with hexane and thereafter by water, dried and re-crystallized from benzene. Yield: 80%, mp 190–192 °C; IR (KBr) ν_{max}: 3421 (NH), 2951 (C–H), 1687 (C=O), 1522 (C=N), 1446 (C=C, arom), 1359 (C–H, arom. bend), 1257 (C–O, C–N), 1229 (C–C): EI-M S (*m*/*z*): 279 (M^+, 14), 263 (12), 235 (16), 222 (31), 221 (51), 194 (11), 208 (26), 163 (3), 145 (100), 137 (5), 120 (32), 119 (72), 91 (62), 65 (19), 57 (26), 56 (23): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.86 (d, 1H, *J* = 8, Ar-H), 8.63 (d, 1H, *J* = 6, Ar-H), 7.83–7.76 (m, 2H, Ar-H), 7.57–7.52 (t, 1H, *J* = 7, Ar-H),7.31 (d, 2H, *J* = 9, Ar-H), 7.18 (t, 1H, *J* = 7, Ar-H), 4.10 (t, 2H, *J* = 6, CH_2), 3.52 (t, 2H, *J* = 6), 2.49(s, 3H, CH_3), 1.18(s, 2H, N–H).

4.1.11. 3-(3-Aminopropyl)-2-(4-methylphenyl) quinazolin-4(3*H*)-one (11)

A mixture of 2-(4-methylphenyl)-4*H*-3,1-benzoxazin-4-one (**4**) and 1,3-diaminopropane (2 ml) in dry pyridine (15 ml) was refluxed with continuous stirring for 6 h. After completion of the reaction as indicated by TLC, the mixture was acidified with few drops of HCl. The product precipitating upon cooling was filtered, washed with hexane and then with water, dried over anhydrous $NaSO_4$ and re-crystallized from benzene. Yield: 91% mp 238–239 °C; IR (KBr) ν_{max}: 3228 (N–H), 3005 (C–H), 1668 (C=O), 1504 (C=N), 1424 (C=C, conj.), 1311 (C–N), 1258 (C–C); EI-MS *m*/*z*: 293 (M^+, 4), 267 (5), 238 (16), 202 (26), 163 (3), 137 (5), 120 (32), 119 (100), 91 (62), 65 (19), 57 (26), 56 (23), ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.86 (d, 1H, *J* = 8), 8.63 (d, 1H, *J* = 6), 7.83–7.76 (m, 3H), 7.57–7.52 (t, 1H, *J* = 7), 7.31 (d, 2H, *J* = 9), 7.18 (t, 1H, *J* = 7), 5.6 (t, 2H, NH), 3.33 (t, 2H, *J* = 6), 2.72 (t, 2H, *J* = 9), 1.79–1.61 (m, 2H).

4.1.12. 2-Phenyl-3-(1, 3-thiazol-2-yl) quinazolin-4(3*H*)-one (12)

A mixture of 2-phenyl-4*H*-3,1-benzoxazine-4-one (0.3 g, 0.001345 mol) and 2-aminothiazole (0.1345 g,) was refluxed in dry pyridine. Yield: 71%, mp 186–189 °C: IR (KBr) ν_{max} cm^{-1}: 1671 (C=O), 11,601 (C=N), 1520 (C=C,), 1301 (C–H, bend), 1245 (C–O), 1234 (C–C), 834, 756, 681 (C–H, arom. bend): EI-MS *m*/*z*: 305 (M+,15), 274 (6), 262 (25), 248 (21), 228 (78), 222 (100), 208 (13), 191 (10), 146 (20), 119 (100), 91 (39), 65 (13): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.99 (d, 1H, *J* = 9), 8.47 (d, 1H, *J* = 9), 8.04–8.019 (m, 2H), 7.65–7.56 (m, 4H), 7.89 (t, 1H, *J* = 9) 7.20 (d, 1H, *J* = 4), 6.88 (d, 1H, *J* = 3).

4.1.13. 3-Amino-2-(4-chlorophenyl)quinazolin-4(3*H*)-one (13)

Yield: 90%, mp 189–190 °C; IR (KBr) ν_{max}: 3212 (N–H), 1769 (C=O), 1660 (C=N, C=C), 1245 (C–O, C–N), 1172 (C–C), 768 (C–Cl); EI-MS *m*/*z*: 273(M^{+2}, 50), 272(53), 271 (M^+, 100), 270 (86), 257 (4), 255 (13), 243 (100), 242 (61), 214 (4), 213 (5), 202 (24), 178 (73), 90 (3), 77 (5), 76 (8), 50 (4); ^{1}H NMR (300 MHz, $CDCl_3$), δ 8.30 (d, 1H, *J* = 6), 7.79–7.77 (m, 4H), 7.57–7.44 (m, 3H), 5.05(b, s, 2H).

4.1.14. 3-Mesityl-2-phenylquinazolin-4(3*H*)-one (14)

2-Phenyl-4*H*-3,1-benzoxazine-4-one (1 g) and 2,4,6-trimethylaniline in dry benzene (10 ml) was refluxed for 8 h with continuous stirring. After completion of the reaction, the reaction mixture was allowed to cool below room temperature. The precipitated solid upon cooling was filtered, washed with hexane, dried and re-crystallized from ethanol. Yield: 73%, mp 210–213 °C; IR (KBr) ν_{max}: 2934 (C–H), 1641 (C=O), 1496 (C=C), 1255 (C–C); EI-MS *m*/*z*: 340 (M^+, 12), 254 (10), 222 (60), 146 (5), 135 (100), 120 (18), 105 (58), 92 (5), 76 (20), 65 (4); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.7 (d, 1H, *J* = 8), 8.02 (d, 1H, *J* = 8), 7.95 (d, 2H, *J* = 8), 7.63–7.42 (m, 5H), 7.28 (t, 1H, *J* = 8), 6.96 (s, 2H), 2.3 (s, 3H), 2.2 (s, 6H).

4.1.15. 3-(2-Hydroxyethyl) quinazolin-4(3*H*)-one (15)

A mixture of anthranilic acid (1.2 mmol), triethylorthoformate (2.0 mmol), monoethanolamin (2 mmol) and *para*-toluene sulfonic acid (5 mmol) was stirred at room temperature under argon atmosphere for 1.5 h. After completion of the reaction as indicated by TLC, the reaction mixture was poured into 5%, aq. $NaHCO_3$ and followed by filtering of the precipitating product. A second crop of the product was collected from the filtrate by extracting with dichloromethane, and the product obtained was dried and re-crystallized from ethanol. Yield: 89%; IR (KBr) ν_{max}: 3250 (N–H), 2825 (C–H), 1675 (C=O), 1589 (C=C), 1450 (C=C), 1275 (C–O), 1250 (C–C); EI-MS *m*/*z*: 190 (22), 171 (4), 160 (10), 159 (12), 147 (100), 146 (690, 145 (12), 130 (46), 129 (32), 119 (10), 105 (2), 102 (17), 92 (4), 77 (27), 76 (15), 51 (6): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (d, 1H, *J* = 8.04), 8.05 (s, 1H), 7.72–7.67 (t, 1H, *J* = 8.4), 7.615 (d, 1H, *J* = 7.8), 7.452–7.398 (t, 1H, *J* = 8.16), 4.1506–4.1184 (t, 2H, *J* = 4.8), 3.99–3.975 (t, 1H, 4.5), 2.978 (b, s, 1H).

4.1.16. 3-(2-Hydroxyethyl)-2-methylquinazolin-4(3*H*)-one (16)

A mixture of anthranilic acid (1.2 mmol), triethylorthoacetate (2 mmol), monoethanolamin (2 mmol) and *para*-toluene sulfonic acid (5 mmol) under argon atmosphere was stirred at room temperature for 1 h. After completion of the reaction, the reaction mixture was poured into water and extracted with dichloromethane. Excess of solvent was evaporated under reduced pressure and the solid left behind was re-crystallized from ethanol–water mixture. Yield: 86%; IR (KBr) ν_{max}: 3250 (O–H), 1664 (C=O), 1534 (C=N, C=C), 1449 (C=C, conj.), 1304 (C–O), 1255 (C–C), 757, 700, 664 (arom. =C–H, bend); EI-MS *m*/*z*: 204 (M^+,28), 185 (9), 173 (13), 161 (59), 169 (100), 159 (7), 146 (5), 145 (10), 143 (13), 119 (7), 118 (7), 116 (9), 92 (2), 90 (5), 77 (13), 76 (8), 50 (3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.190 (d, 1H, *J* = 8), 7.38–7.682 (t, 1H, *J* = 7.9), 7.631 (d, 1H, *J* = 7.3), 7.446–7.396 (t, 1H, *J* = 7.7), 4.316–4.282 (t, 2H, *J* = 5.07), 4.026–3.992 (t, 2H, *J* = 5.26), 3.22 (b, s, 1H), 2.72 (s, 3H).

4.1.17. 2-Aminobenzoyle-*N*-[3-(4-oxo-2-phenylquinazolin-3(4*H*)-yl) propyl]benzamide (17)

2-Phenyl-4*H*-3,1-benzoxazin-4-one (3.76 g, 28.8 mmol) and 1,2-diaminopropane (2 ml) in 30 ml dry benzene was refluxed. Yield: 81%, mp 90 °C; IR (KBr): 3451 (N=C–OH), 3234 (N–H), 1662 (C=O), 1528 (C=O, conj.), 1444 (–C=C, conj.) 1255 (C–N, C–O), 1164 (C–C), 757, 706, 669 (=C–H, arom. bend): EI-MS *m*/*z*: 502 (M^+, 31), 425 (5), 397 (6), 263 (10), 262 (27), 254 (9), 249 (38), 236 (31), 235 (100), 224 (78), 223 (51), 120 (33), 119 (10), 105 (89), 77 (38), ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.8 (s, 2H, NH), 8.61 (d, 2*H*, *J* = 7), 8.02 (d, 2H, *J* = 9), 7.99 (d, 4H, *J* = 9), 7.557.45 (m, 8H), 6.89 (t, 2H, *J* = 6), 4.01 (t, 2H, 5.3), 3.58 (t, 2H, *J* = 6), 2.12 (m, 2H).

4.1.18. *N*-(2-Phenyl-4-oxoquinazolin-3(4*H*)-yl)acetamide (18)

3-Amino-2-phenyl-4(3*H*)-quinazolinone (0.2 g, 0.85 mmol), triethylorthoacetate (0.37 ml) and glacial acetic acid (1.0 ml) was

reflux with continuous stirring for 5 h. After completion of the reaction, the reaction mixture was poured into ice-cold water, the product separated as a white crystalline solid which was filtered, washed with water and re-crystallized from ethanol. Yield: 83%, mp 132–133 °C; IR (KBr), ν_{max}: 3335 (b, 2° N–H, str.), 3233 (s, =C–H, str.), 2868 (s, C–H, str.), 1840 (w, C=C, overtone), 1720 (s, C=O, str.), 1659 (s, w, C=C, str.), 1431 (m, s, C–C-H, bend), 1217 (m, s, C–O, str.), 1172 (s, C–C, str.), 813 (s, m, C–H, bend, arom. region), 765 (s, C–H, bend, arom. region), 685 (s, C–H, bend); EI-MS *m*/*z*:307 (M^+, 2), 279 (85), 237 (23), 202 (5), 105 (100), 77 (45), 51 (10): ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm: 9.02 (d, 1H, *J* = 8), 8.175 (d, 2H, *J* = 8), 7.92 (d, 1H, *J* = 7.8), 7.54 (m, 3H), 7.24 (m, 2H), 3.7 (q, 2H), 2.65 (s, 3H), 1.24 (t, 3H).

4.1.19. 4-Methyl-*N*-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)benz amide (19)

2-Methyl 3-aminoquinazolin-4(3*H*)-one (0.03 g) and 4-methylbenzoylchloride in dry pyridine (10 ml) was refluxed for 30 min. After completion, the reaction mixture was poured into ice-cold water. The product precipitating was filtered, washed with water and re-crystallized from ethanol. Yield: 83%, mp 193–195 °C; EI-MS *m*/*z*: 293 (M^+, 3), 202 (11), 174 (28), 160 (7), 159 (100), 145 (78), 132 (8), 120 (31), 119 (61), 105 (54), 77 (11), 65 (5), 54(3); ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.2 (s, 1H), 8.4 (d, 1H, *J* = 7.92), 8.35 (t, 1H, *J* = 8.5), 7.85 (t, 1H, *J* = 8.5), 7.71 (d, 1H, *J* = 8.2), 7.53 (t, 1H, *J* = 8), 7.41 (d, 2H, *J* = 7.92), 2.54 (s, 3H), 2.31 (s, 3H).

4.1.20. *N*-(2-Methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide (20)

A mixture of 3-amino-2-methylquinazolin-4(3*H*)-one (0.5 g, 0.00285 mol) and acetyl chloride was stirred in dry pyridine (10 ml) for 1 h. After completion of the reaction, the reaction mixture was poured into ice-cold water and the product precipitating was filtered, washed and re-crystallized from ethanol. Yield: 91%, mp 172–174 °C: IR (KBr) ν_{max}: 2954 (C–H), 1741 (C=), 1706 (C=O), 1483 (C=C–C), 1462 (C–H, bend), 1238 (C–C), 1079, 1002, 971 (C–H, bend, arom.): EI-MS *m*/*z*: 217 (M^+, 13), 175 (100), 160 (14), 146 (73), 118 (9), 117 (19), 90 (18), 77 (23), 76 (29), 63 (9), 62 (11), 51 (13) ^{1}H NMR (300 MHz) δ (ppm): 11.3 (b, s, 1H), *J* = 10), 8.021 (d, 1H, *J* = 10), 2.48 (s, 2H), 2.1 (s, 3H): ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 169, 160, 158, 157, 144, 137, 135, 128, 127, 126, 120, 117, 21, 18.

4.1.21. 2-Chloro-*N*-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetam ide (21)

A mixture of 3-amino-2-methylquinazolin-4(3*H*)-one (0.1 g, 0.00057 mol) and chloroacetylchloride (0.1 ml) in 10 ml dry benzene were refluxed under argon atmosphere with continuous stirring for 5 h. The reaction mixture was then cooled, and excess of solvent was evaporated under reduced pressure, the resulting solid was washed with water, dried and re-crystallized from benzene. Yield: 41.9%, mp 196–199 °C: IR (KBr) ν_{max}: 3492 (N–H), 2929 (C–H), 1638 (C=O), 1598 (C=O), 1473 (C=C–C), 1280 (C–C=C), 774 (C–Cl): EI-MS *m*/*z*: 253 (M^{+2}, 13), 251 (M^+, 20), 202 (54), 175 (81), 161 (29), 159 (100), 146 (71), 118 (10), 117 (19), 90 (16), 77 (23), 76 (31), 62 (11), 51 (14): ^{1}H NMR (300 MHz) δ (ppm): 11.3 (s, b, 1H), 8.21 (d, 1H, *J* = 9), 8.15 (d, 1H, *J* = 10), 7.9–7.87 (t, 1H, *J* = 10, *J* = 8.5), 7.58–7.55 (t, 1H, *J* = 10, *J* = 8.5), 3.84 (s, 2H), 2.54 (s, 3H).

4.1.22. *N*-(2-Methyl-4-oxoquinazolin-3(4*H*)-yl)benzamide (22)

3-Amino-2-methylquinazolin-4(3*H*)-one (0.5 g, 0.00285 mol) was dissolved in dry pyridine (10 ml) and stirred. To the stirred solution benzoylchloride (1 ml) was added drop wise and stirring was continued for the next 1 h. After completion, the reaction mixture was poured into water and the product separated out after a while was filtered, washed with water and re-crystallized from ethanol. Yield: 78%, mp 102–103 °C; IR (KBr) ν_{max}: 3165 (N–H), 2921 (C–H), 1735 (C=O), 1696 (C=O), 1499 (C=C), 1417 (C=C–C), 1377 (C–H, bend), 1256 (C–C=C): EI-MS *m*/*z*: 279 (M^+, 8), 167 (8), 149 (22), 122 (40), 106 (6), 105 (100), 84 (8), 77 (52), 76 (7), 71 (8), 66 (14), 57 (17), 56 (10), 52 (40): ^{1}H NMR (300 MHz) δ (ppm): 11.85 (s, b, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 172, 134, 130, 129, 128, 77.6, 77, 76.

4.1.23. 3-({(1*E*)-[4-(Dimethylamino) phenyl]methylene}amino)-2-phenylquinazolin-4(3*H*)-one (23)

Few drops of H_2SO_4 was added to 4-*N*,*N*-dimethyl benzaldehyde (0.0342 g, 0.00252 mol). The mixture was dissolved in absolute ethanol and stirred for 10–15 min. 2-Phenyl 3-aminoquianzolin-4(3*H*)-one (0.03 g, 0.000126 mol) was then added to the stirred solution and the combined mixture was refluxed with contentious stirring for 1 h. After completion of the reaction, the mixture was poured into $NaHCO_3$ solution. The precipitating product was filtered, washed with cold water and re-crystallized from ethanol. Yield: 67%, mp 163 °C; IR (KBr) ν_{max}: 2927 (CH), 1666 (C=O), 1601 (C=N), 1425 (C–H, bend), 1378 (C–H, bend), 1259 (C–O), 1174 (C–C), 830, 763, 688 (C–H, arom. bend): FAB-MS: (+ve, −ve) 369, 367: EI-MS *m*/*z*: 223 (57), 222 (4), 146 (42), 145 (20), 130 (6), 120 (17), 119 (10), 115 (9), 105 (100), 92 (9), 77 (79), 69 (6), 51 (13): ^{1}H NMR (300 MHz, CD_3OD) δ ppm: 9.82 (s, 1H), 8.62 (d, 1H, *J* = 8), 7.96–7.85 (m, 2H), 7.72–7.65 (m, 3H), 7.51–7.43 (m, 5H), 6.95 (d, 2H, *J* = 8), 2.95 (s, 6H).

4.1.24. 3-({(1*E*)-[4-(Dimethylamino) phenyl]methylene}amino)-2-methylquinazolin-4(3*H*)-one (24)

Few drops of H_2SO_4 was added to 4-*N*,*N*-dimethyl benzaldehyde (0.0342 g, 0.00252 mol). The mixture was dissolved in absolute ethanol and stirred for 10–15 min. 2-Methyl 3-aminoquianzolin-4(3*H*)-one (0.026 g, 0.00014857 mol) was then added to the stirred solution. The mixture was refluxed with contentious stirring for 1 h. After completion of the reaction, the mixture was poured into $NaHCO_3$ solution. The product precipitating out was filtered, washed with cold water and re-crystallized from ethanol. Yield: 75%, mp 166–168 °C; IR (KBr) ν_{max} cm^{-1}: 3068 (C–H), 1666 (C=O), 1601 (C=N),1425 (C–H, bend), 1259 (C–O), 1231 (C–N), 1174 (C–C): EI-MS *m*/*z*: 306 (10), 183 (57), 160 (87), 159 (100), 147 (34), 146 (18), 145 (4), 136 (2), 133 (19), 130 (11), 121 (18), 120 (56) 119 (23), 106 (68), 105 (100), 92 (9), 77 (10), 76 (7), 63 (11), 52 (13); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 9.82 (s, 1H, weak), 8.62 (d, 1H, *J* = 8), 7.9 (d, 1H, *J* = 10), 7.51–7.42 (m, 2H), 7.19 (d, 2H, *J* = 10), 6.95 (d, 2H, *J* = 8), 2.94 (s, 6H, N–CH_3), 2.54 (s, 3H, CH_3).

4.1.25. 3-{[(1*E*)-(3-Chlorophenyl)methylene]amino}-2-phenyl quinazolin-4(3*H*)-one (25)

3-Chlorobenzaldehyde (0.01 ml, 0.00012658 mol) was dissolved in absolute ethanol containing few drops of H_2SO_4 and stirred for 10–15 min. 3-Amino-2-phenylquinazolin-4(3*H*)-one (0.03 g, 0.00012658 mol) was then added to the stirred solution. The obtained mixture was refluxed with contentious stirring for 1 h. After completion of the reaction, the mixture was poured into $NaHCO_3$ solution. The product precipitated out was filtered, washed with cold water and re-crystallized from ethanol. Yield: 75% mp 210–212 °C; R (KBr) ν_{max}: 1601 (C=O), 1529 (C=N), 1444 (C=C, arom.), 1233 (C–C), 919, 840, 757 (C–H, arom. bend), 709 (C–Cl): EI-MS *m*/*z*: 359 (12), 325 (12), 313 (21), 298 (14), 249 (45), 222 (67), 221 (81), 208 (17), 120 (13), 106 (12), 105 (100) 92 (7), 67 (16), 54 (5): ^{1}H NMR (400 MHz) δ (ppm):9.3 (s, 1H), 8.35 (d, 1H, *J* = 7.8), 8.23–8.21 (m, 2H), 7.96–7.94 (m, 3H), 7.7–7.58 (m, 5H), 7.48 (d, 1H, *J* = 8), 6.67 (s, 1H).

4.1.26. 3-{[(1*E*)-(2-Hydroxynaphthalen-1-yl)methylene]amino}-2-phenylquinazolin-4(3*H*)-one (26)

2-Hydroxynephthaldehyde (0.07 g, 0.000329 mol) was dissolved in absolute ethanol (10 ml) and few drops of H_2SO_4 was added as catalyst and thereafter stirred at room temperature for 30 min. 3-Amino-2-phenylquinazolin-4(3*H*)-one (0.01 g, 0.000329 mol) was added to the stirred solution and the mixture was refluxed for 30 min. The precipitate appearing upon cooling was washed with 5% aq. $NaHCO_3$ and re-crystallized from ethanol. The pure compound was obtained as yellowish needles like crystals. Yield: 77.65%, mp 258.9–260 °C: IR (KBr) ν_{max}: 3436 (O–H), 1719 (C=O), 1575 (C=N), 1512 (C=C), 1480 (C=C–C), 1303 (C–H, bend), 1005, 826, 752 (C–H, arom. bend): EI-MS *m/z*: 391 (M), 222 (5), 186 (23), 169 (9), 128 (15), 120 (22), 119 (10), 115 (13), 105 (100), 92 (16), 77 (85), 65 (10), 51 (15); ^{1}H NMR (300 MHz) δ (ppm): 11.6 (s, 1H), 10.5 (s, 1H), 8.38 (d, 1H, *J* = 9), 8.28 (d, 1H, *J* = 9), 8.05 (d, 2H, *J* = 9), 7.55–7.49 (m, 5H), 7.39–7.29 (m, 4H), 7.04 (s, 1H), 6.79 (t, 1H, *J* = 9).

4.1.27. 3-{[(1*E*)-(4-Fluorophenyl)methylene]amino}-2-phenylquinazolin-4(3*H*)-one(27)

4-Flourobenzaldehyde (0.04 g, 0.000329 mol) was dissolved in absolute ethanol (10 ml) and 2–3 drops of H_2SO_4 was added as catalyst, and thereafter stirred at room temperature for 30 min. 3-Amino-2-phenylquinazolin-4(3*H*)-one (0.01 g, 0.000329 mol) was added to the stirred solution and the mixture was refluxed for 5 h. The precipitate appearing upon cooling was washed with 5% aq. $NaHCO_3$ and re-crystallized from ethanol and the pure compound was obtained as yellowish needles like crystals. Yield: 86%, mp 163–164 °C; EI-MS *m/z*: 343 (M^+, 41), 265 (71), 248 (13), 221 (81), 208 (37), 180 (45), 145 (100), 120 (39), 105 (71), 77 (12), 76 (25), 65 (5), 54 (3); ^{1}H NMR (300 MHz) δ (ppm): 9.31 (s, 1H), 8.43 (t, 1H, *J* = 7.8), 8.25 (d, 1H, *J* = 8), 8.2–7.96 (m, 2H), 7.83 (m, 2H), 7.76–7.69 (m, 2H), 7.68 (t, 2H, *J* = 8.2), 7.46(d, 1H, *J* = 7.8).

4.1.28. 2-(4-Oxo-2-methylquinazolin-3(4*H*)-yl) guanidine (28)

A mixture of 2-methyl 3-aminoquianzolin-4(3*H*)-one (0.026 g, 0.00014857 mol) and urea (0.018 g, 0.000296 mol) in absolute ethanol (10 ml), catalyzed with 2 ml glacial acetic acid was refluxed for 3 h. After completion of the reaction, the mixture was poured into ice-cold water, the precipitated product was filtered, washed with water and re-crystallized from ethanol. Yield: 65.5%, mp 168–172 °C; EI-MS *m/z*: 217 (M^+, 21), 201 (31), 186 (7), 160 (78), 159 (100), 146 (71), 120 (42), 105 (31), 77 (7), 76 (12), 65 (3), 54 (5): ^{1}H NMR (300 MHz) δ (ppm): 8.02 (d, 1H, *J* = 8), 7.91 (t, 1H, *J* = 7.6), 7.87 (d, 1H, *J* = 8), 7.69 (t, 1H, *J* = 8.2), 4.56 (s, 4H), 2.54 (s, 3H).

4.1.29. Ethanimidic acid, *N*-[4-oxo-2-phenyl-3(4*H*)-quinazolinyl]-, ethyl ester (29)

A mixture of 3-amino-2-phenyl-4(3*H*)-quinazolinone (0.2 g, 0.85 mM), triethylorthoacetate (0.37 ml) and 0.5 ml of glacial acetic was reflux with continuous stirring for 5 h. After completion of reaction, the reaction mixture was poured into ice-cold water, and the product separated as a white crystalline solid. The crystalline solid was filtered, washed with water and re-crystallized from ethanol to give (83%) of the compound as white crystals; yield: 76%, mp 132–133 °C.; IR (KBr) ν_{max}: 3335 (b, 2° N–H, str), 3233 (s,=C–H, str), 2868 (s, C–H, str), 1840 (w, C=C, overtone), 1720 (s, C=O, str), 1659 (s, w, C=C, str), 1431 (m, s, C–C–H, bend), 1217 (m, s, C–O, str.), 1172 (s, C–C, str), 813 (s, m, C–H, bend, arom. region), 765 (s, C–H, bend, arom. region), 685 (s, C–H, bend): EI-MS *m/z*:307 (M^+, 2), 279 (85), 237 (23), 202 (5), 105 (100), 77 (45), 51 (10): ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm): 9.02 (d, 1H, *J* = 8), 8.175 (d, 2H, *J* = 8), 7.92 (d, 1H, *J* = 7.8), 7.54 (m, 3H), 7.24 (m, 2H), 3.7 (q, 2H,), 2.65 (s, 3H), 1.24 (t, 3H).

4.1.30. *N*-(4-Oxo-2-phenyl-3(4*H*)-quinazolininyl)-*N*-(3-nephthyl) acetamidine (30)

Ethanimidic acid, compound **29** (0.2 g, 0.65 mmol) and 2-naphthylamine (0.093 g, 0.65 mmol) were fused to melt and then added 1–2 ml of glacial acetic acid and refluxed for 4–5 h. Completion of reaction was monitored by TLC. The reaction product was evaporated under reduced pressure and the remaining mixture was allowed to cool to room temperature. The solid formed upon cooling was filtered, washed with water and re-crystallized from ethanol to yield the target amidine: yield (66%): mp 218–220 °C; IR (KBr) ν_{max} cm^{-1}: 3241 (N–H, str.), 2933 (C–H, str), 2866 (C–H, str.), 1651 (C=O, str.), 1520 (C=C, str.), 1417 (C–H, bend), 1203 (C–C, str.), 975, 885, 780 (C–H, bend, arom.); EI-MS *m/z*: 404 (M^+, 23), 376 (8), 327 (42), 299 (4), 261 (2), 230 (2), 222 (71), 168 (12), 127 (22) 105 (100), 77 (70); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.53 (s, 1H, N–H), 7.95 (d, 1H, *J* = 8), 7.83 (t, 1H, *J* = 8.3), 7.78 (t, 2H, *J* = 8), 7.71 (d, 2H, *J* = 7.6), 7.65–7.61 (m, 6H), 7.43–7.39 (m, 2H), 7.27 (t, 1H, *J* = 8.3), 7.12 (d, 1H, *J* = 7.89), 2.35 (s, 3H).

4.1.31. General method for the synthesis of compounds 31–33

A mixture of appropriate 2-alkyl/aryl-aminoquinazolin-4(3*H*)-one (0.28 mmol) and $KMnO_4$ (1.12 mmol) in 10 ml water was refluxed for 3–5 h. After completion of the reaction (monitored by TLC), the reaction mixture was hot filtered and the filtrate was allowed to cool. The precipitated product was filtered, dried and re-crystallized from water–ethanol mixture to give the compounds (**31**–**33**).

4.1.32. 2-Ethylquinazolin-4(3*H*)-one (31)

IR (KBr): 3170 (NH), 3065 (C–H), 2925 (C–H), 1667 (C=O), 1603 (C=N), 1510 (C=C), 1471 (C=C arom.), 1451 (C=C, arom), 1240 (C–C), 1144 (C–O), 786 (C–C, bend, arom.), 692 (C–C, bend, arom.): FAB-MS *m/z*: 161 (M+1), 159 (M−1); EI-MS *m/z*: 160 (M^+, 100), 145 (21), 120 (19), 92 (23), 76 (10), 64 (11): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.16 (d, 1H, *J* = 7.6 Hz), 7.78 (t, 1H, *J* = 7.1 Hz), 7.6 (d, 1H, *J* = 7.8 Hz), 7.48 (t, 1H, *J* = 7.1 Hz), 2.4 (s, 3H).

4.1.33. 2-(4-Chlorophenyl) quinazolin-4(3*H*)-one (32)

Yield: 72%, mp 303 °C; IR (KBr) ν_{max} cm^{-1}: 3312 (N–H), 1705 (C=O), 1548 (C=N), 1378 (C–H, bend), 1250 (C–C): EI-MS *m/z*: 258 (M^{+2}, 12), 256 ($M.^+$, 52), 178 (4), 149 (13), 138 (9), 119 (100), 113 (3), 111(12), 102 (5), 94 (14), 91 (4), 90 (13), 77 (5), 76 (8), 57 (8): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.80 (s, 1H), 8.28 (d, 1H, *J* = 11 Hz), 8.04 (d, 2H, *J* = 11.07 Hz), 7.82 (d, 1H, *J* = 10 Hz), 7.75 (d, 1H, *J* = 11 Hz), 7.54 (d, 2H, *J* = 11 Hz), 7.42 (d, 1H, *J* = 11.2 Hz).

4.1.34. 2-(4-Methylphenyl) quinazolin-4(3*H*)-one (33)

Yield: 73%, mp 240–242 °C; IR (KBr) ν_{max} cm^{-1}: 3347 (NH), 2924 (C–H), 2796 (C–H), 1672 (C=O), 1610 (C=N), 1470 (C=N), 1373 (C–C, bend, arom.), 1231 (C–O), 1188 (C–C), 769 (C–C, bend, arom.), 681 (C–C, bend, arom): EI-MS *m/z*: 236 (M^+, 57), 119 (100), 91 (25), 90 (25), 76 (13), 63 (14): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.11 (s, 1H), 7.92 (d, 1H, *J* = 9 Hz), 7.69 (m, 3H), 7.20 (m, 4H), 2.348 (s, 3H, CH_3).

4.1.35. Ethyl (4-oxo-3,4-dihydroquinazolin-2-yl)acetate (34)

A stream of dry hydrogen chloride was passed through a mixture of methylanthranilate and ethylcyanoacetae in dry 1,4-dioxane solvent for 6–12 h. After completion of the reaction as indicated by TLC, the reaction mixture was basified with 10% $NaHCO_3$. The product was extracted with DCM and dried over anhydrous $NaHSO_4$. Excess of solvent was evaporated under pressure and products were purified by re-crystallization from water–ethanol mixture. Yield: 65, mp 155–157 °C; IR (KBr) ν_{max}: 3352(N–H), 1664 (C=O), 1530 (C=N), 1445 (C=C), 1328 (C–H, bend), 1235

(C–N, C–C), 1036, 950, 926 (C–H, arom. bend): EI-MS *m/z*: 232 (M^+, 62), 160 (100), 119 (37), 89 (39), 77 (28), 60 (42), 55 (21). ^{1}H NMR ($CDCl_3$, 400 MHz, δ (ppm): 8.26 (d, 1H, J = 7.8 Hz), 7.76 (m, 2H), 7.49 (t, 1H, J = 7.1 Hz), 4.26 (q, 2H, J = 7.36 Hz), 3.86 (s, 1H), 1.34 (t, 3H, J = 7.17 Hz).

4.1.36. 3-(Isopropylideneamino)-2,2-dimethyl-2,3-dihydroquinazolin-4(1*H*)-one (35)

0.5 g 2-aminobenzohydrazide (**26**) in excess of acetone was stirred at room temperature overnight on a normal silica gel. After completion, the reaction mixture was filter through sintered glass funnel, the solvent was evaporated under reduced pressure and the remaining solid was washed with water and re-crystallized from dilute ethanol to afford 92% of the target compound. mp 245–248 °C; IR (KBr) ν_{max}: 3306 (N–H), 2973 (C–H), 1627 (C=O), 1509 (C=N), 1489 (C=C), 1357 (C–H, bend), 1273 (C–N): EI-MS *m/z*: 231 (M^+, 30), 217 (15), 216 (100), 191 (6), 174 (3), 161 (17), 160 (89), 132 (4), 121 (4), 117 (10), 91 (5), 87 (4), 85 (22), 77 (3), 52 (19); ^{1}H NMR (300 MHz) δ (ppm): 7.89 (d, 1H, J = 8), 7.29 (t, 1H, J = 8), 6.839 (t, 1H, J = 7.5), 6.618 (d, 1H, J = 8), 4.161 (s, b, 1H),2.15 (s, 3H, CH_3), 1.878 (s, 3H, CH_3).

4.1.37. 2-Phenyl-3-{[(1*E*)-phenylmethylene] amino}-2,3-dihydroquinazolin-4(1*H*)-one (36)

2-Aminobenzohydrazide (0.2 g, 0.00132 mol) and redistilled benzaldehyde (0.3 g, 0.00264 mol) in 10 ml dry methanol was heated under reflux for 3 h. When the reaction was completed, methanol was allowed to cool and was poured into ice-cold water, the precipitating product was filtered, dried and re-crystallized from ethanol to 69.3% of the target compound. Yield: 69.3%, mp 179 °C; IR (KBr) ν_{max}: 3250 (N–H), 1642 (C=O), 1520 (C=N), 1456 (C=C), 1395 (C–H, bend), 1250 (C–C), 1033, 966 (C–H, arom. bend): EI-MS *m/z*: 328 (10), 327 (8), 250 (12), 139 (11), 224 (24), 223 (60), 209 (23), 208 (100), 181 (8), 180 (32), 179 (5), 167 (6), 163 (6), 161 (9), 160 (16), 152 (16), 151 (6), 149 (13), 148 (10), 147 (67), 132 (5), 130 (9), 121 (6), 120 (31), 119 (21), 111 (7), 109 (5), 105 (21), 104 (17), 97 (10), 92 (13), 90 (10), 83 (9), 77 (27), 71 (11), 69 (10),47 (10): ^{1}H NMR (300 MHz) δ (ppm): 9.59 (s, b, 1H), 8.10 (d, 1H, J = 8), 7.602 (t, 2H, J = 8), 7.47 (t, 2H, J = 8), 7.37–7.30 (m, 10H), 6.28 (s, 1H).

4.1.38. 2-Phenyl-3-[(*E*)-phenyldiazenyl]-1,2,3,4-tetrahydro-10*H*[1,2,4]triazino[6,1*b*]-quinazolin-10-one (37)

Benzaldehyde (0.00252 mol) was added to a stirred solution of 3-amino-2-(hydrazinomethyl) quinazolin-4(3*H*)-one (0.0258 g, 0.000126 mol) in absolute ethanol and the combined mixture was refluxed for 2 h. After completion of the raection, the raection mixture was poured into ice-cold water. The precipitated product was filtered, washed with water and re-crystallized from ethanol. Yield: 73%, mp 171–173 °C**;** EI-MS *m/z*: 282 (M^+, 2), 281 (7), 368 (27), 293 (15), 278 (12), 264 (21), 174 (18), 162 (12), 161 (38), 160 (100), 146 (100), 120 (12), 119 (10), 105 (73), 77 (8), 76 (42), 65 (4), 54 (5); ^{1}H NMR (300 MHz) δ (ppm): 8.21 (d, 1H, J = 7.8), 8.17 (d, 1H, J = 8.2), 8.01 (t, 1H, J = 7.8), 7.86–7.71 (m, 6H), 7,63 (t, 1H, J = 8.6), 7.52–7.46 (m, 2H), 7.21 (m, 1H), 4.76 (s, 1H), 4.38 (s, 2H, CH_2).

4.2. Binding studies and enzyme kinetics

4.2.1. Chemicals and the enzyme (TLN)

Chemicals used for binding assays and kinetics studies were purchased from Sigma–Aldrich (US). Three-times crystallized TLN (activity ⩾7000 units/mg) were from CalBioChem (E-Merck, Germany). According to the manufacture's instructions the lyophilized protein was first reconstituted in 42% glycerol adjusted to pH 8.0 with 0.01 N NaOH, containing 0.005% Triton® X-100.

4.2.2. In vitro assay based on chemical arrays

The steady-state enzyme assays were performed at 25 °C using the spectrophotometric method of Feder and Schuck.[52]

A 96-well microplate was used to prepare the chemical array. The combinatorially synthesised compounds were dispensed on the array surface with the aid of DMSO. The TLN activity was determined by following the decrease in absorption at 346 nm due to enzymatic hydrolysis of the substrate FaGLa. Initial velocities were determined for ⩽10% of the reaction. Stock solutions of Tris (50 mM), NaBr (2.5 M), and $CaCl_2$ (10 mM), pH 7.0 were prepared and stored at 4 °C. A stock solution of FaGLa was prepared in dimethyl formamide (DMF), and diluted with buffer to a final concentration of 0.1 M Tris, 0.1 M NaBr, and 2.5 mM $CaCl_2$, pH 7.0 (final concentration of DMF, 2.5%).

The total volume of the reaction mixture was 200 μl, in a final enzyme concentration of 50 nM, and with a substrate concentration of 1.0 mM. The enzyme and compounds were incubated for 15 min at 25 °C in a temperature-regulated 96-well microplate. Initial velocities after adding the substrate were determined for ⩽10% reaction in duplicate for each inhibitor concentrations. Control (solvent) readings were taken in six replicates during each experiment.

Preliminary inhibition studies were performed in duplicate arrays and active compounds found from the preliminary array-based screening were run in regular manner at three different concentrations (0.5, 0.05, and 0.005 mM) to obtain dose-dependent curves. Percent inhibition and median inhibitory concentration (IC_{50}) were then calculated.

4.2.3. Enzyme kinetic studies

Enzyme kinetic studies were performed for compounds identified as prominent TLN inhibitors during the initial inhibition studies. The obtained results were used to determine the K_i values. All methods were similar to initial studies (described above) except that multiple concentrations of the substrate (FaGLa) were used (2, 1, 0.75, and 0.5 mM). The kinetic parameters (V_{max} and K_m) were calculated using Enzyme Kinetic™ module of SigmaPlot™ version 10, integrated with SigmaStat™. The enzyme kinetics was based on inhibition of ⩽10% of the reaction.K_i values were determined from IC_{50} of the inhibitors values using the Cheng–Prusoff relation.[45–47]

$$K_i = IC_{50}/(1 + S/K_m) \quad (1)$$

4.3. Molecular docking

Molecular docking of the compounds identified as TLN inhibitors (Table 1) was performed as previously described[51] using the Internal Coordinate Mechanics (ICM) program from Molsoft (http://www.molsoft.com/).

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