

Design, docking analysis, identification, and synthesis of novel 3-(((substituted phenyl) amino)methyl)-2-methylquinazolin-4(3H)-one compounds to fight tuberculosis

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Summary

In this study, a series of novel scaffold-based 3-(((substituted phenyl)amino)methyl)-2-methylquinazolin-4(3H)-one compounds, 3a-3r, was synthesized, characterized, and screened for its *in vitro* activity against the H37Ra strain of *Mycobacterium tuberculosis*. A number of analogs were found to have highly potent anti-tuberculosis activity. Compound 3m in particular had potent activity equal to that of the standard anti-tuberculosis drug rifampicin. New leads can be generated with the model developed in this study and this model will be optimized with the eventual goal of preparing new anti-tuberculosis agents.

Keywords: Quinazoline, amines, antimicrobial activity, antituberculosis activity

1. Introduction

Tuberculosis (TB) is one of the most common infectious diseases. Caused by *Mycobacterium tuberculosis*, TB kills two million people every year and it continues to be a major cause of morbidity and mortality all over the world. About one-third of the world's population is currently infected with TB (1). If the present trend continues, tuberculosis is likely to claim more than 30 million lives within the next decade. Five percent of all TB cases are now estimated to be multi-drug-resistant TB. This form of TB is resistant to the first-line drugs streptomycin, isoniazid, ethambutol, pyrazinamide, and rifampicin and to at least one of the injectable second-line drugs, contributing to the resurgence of the disease (2,3). The overall incidence of TB in HIV-positive patients is 50 times that of the rate for HIV-negative individuals (4-6). Hence, there is an urgent need to discover and develop new anti-TB

agents that target novel biochemical pathways and to treat drug-resistant forms of the disease. Quinazolin-4(3H)-one and related quinazolines are classes of fused heterocycles that are of considerable interest because of the diverse range of their biological properties. Quinazolin-4(3H)-one with a substituted group at position 3 has diverse therapeutic activities including antibacterial (7), antifungal (8), antimalarial (9), anticancer (10), antiinflammatory (11), anticonvulsant (12), and analgesic (13) activity. Quinazoline and its derivatives with a different pharmacophore group each have different modes of action in the treatment of TB. In addition, a survey of the literature revealed that quinazoline and quinazolinone systems have yielded numerous derivatives with anti-TB activity (14). Attention has been drawn to the earlier finding that some quinazoline-based compounds are lipophilic since interactions between ligands and target molecules and permeability are important parameters that determine drug action (15). In the search for novel scaffold-based antimycobacterial agents, a library of quinazolin-4(3H)-one derivatives that was constructed by this Laboratory was screened for action against the drug-susceptible H₃₇Ra strain of *M. tuberculosis*. The action of these compounds was compared to that of the standard anti-TB drugs rifampicin and isoniazid (INH).

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2. Materials and Methods

2.1. Materials

Synthetic starting material, reagents, and solvents were of analytical grade or of the highest quality commercially available. Chemicals were purchased from Aldrich Chemical Co., Merck Chemical Co., and Biotium, Inc. (Ambala, Haryana, India) and were dried whenever necessary. Melting points were determined in open capillary tubes and are uncorrected. Infrared (IR) spectra were recorded with KBr pellets (ABB Bomem FT-IR spectrometer MB 104 from ABB Limited, Bangalore, India). Proton nuclear magnetic resonance (¹H NMR) spectra (Bruker NMR spectrometer, Punjab University, Chandigarh, India) were recorded with tetramethylsilane as an internal reference. Mass spectral data were recorded with a quadrupole mass spectrometer (Shimadzu GC MS QP 5000, Punjab University, Chandigarh, India) and microanalyses were performed using a *vario* EL V300 elemental analyzer (Analysensysteme GmbH, Chennai, India). The purity of the compounds was determined with thin-layer chromatography on aluminium plates pre-coated with SiO₂ gel (HF₂₅₄, 200 mesh) (E. Merck, Ambala, Haryana, India). IR, ¹H-NMR, mass spectral data, and elemental analyses were consistent with the proposed structures.

2.2. General procedures

A mixture of anthranilic acid (1.37 g, 0.01 mol) and acetic anhydride (10.2 mL, 0.1 mol) was refluxed over a gentle flame for 1 h. Excess acetic anhydride was distilled off under reduced pressure and the residue was dissolved in petroleum ether. The residue was left to stand for 1 h. 2-methyl-4*H*-benzo[1,3]oxazin-4-one

1 was obtained as a light brown solid, and this solid was filtered and dried. A mixture of 2-methyl-4*H*-benzo[1,3]oxazin-4-one **1** (0.01 mol) and ammonium acetate (0.01 mol) was combined in an oil bath for 2 h. The mixture was poured into an ice/water mixture and stirred. The precipitate that separated out was filtered, washed, dried, and then recrystallized from ethanol, yielding 2-methylquinazolin-4(3*H*)-one **2** as beige crystals. The appropriate substituted aromatic amines (0.02 mol) were slowly added to a solution of **2** (0.01 mol) in glacial acetic acid (50 mL) containing 37% formalin (1 mL). The reaction mixture was refluxed in a water bath for 1-3 h. The reaction mixture was concentrated to approximately half its initial volume, and the resulting precipitate was recrystallized from ethanol to yield a pure form of 3-(((substitutedphenyl)amino)methyl)-2-methylquinazolin-4(3*H*)-one compounds **3a-3r**. Table 1 shows the physical data for the synthesized compounds.

2.3. In silico study

Data on the synthesized compounds that docked in the crystal structure of DNA gyrase were obtained from the Protein Data Bank (PDB entry code 1KIJ2) and analyzed using Auto Dock 4.2. All polar hydrogen atoms were added and partial charges were placed with the help of the GROMACS package. The energy of the molecule was minimized by keeping heavy atoms fixed at their initial crystal coordinates and the added hydrogen atoms were allowed to move. Minimization was performed under a vacuum. Electrostatic interactions were calculated using the cut-off method. Solvation parameters were added using the ADDSOL option in Auto Dock 4.2. Default values for atomic solvation parameters were used throughout the calculations. Grid maps of the protein were calculated using the program

Table 1. Physical data on 3-(((substituted phenyl)amino)methyl)-2-methylquinazolin-4(3*H*)-one 3a-3r

Compounds	R1	Mol. formula	% yield	M.p (°C)	R _f ^a
3a	<i>o</i> -F	C ₁₆ H ₁₄ FN ₃ O	70	245-247	0.56
3b	<i>m</i> -F		76	215-217	0.51
3c	<i>p</i> -F		81	212-214	0.66
3d	<i>o</i> -Br	C ₁₆ H ₁₄ BrN ₃ O	79	223-225	0.72
3e	<i>m</i> -Br		72	241-243	0.74
3f	<i>p</i> -Br		74	262-265	0.55
3g	<i>o</i> -Cl	C ₁₆ H ₁₄ ClN ₃ O	73	211-213	0.62
3h	<i>m</i> -Cl		70	210-212	0.74
3i	<i>p</i> -Cl		86	272-274	0.83
3j	<i>o</i> -NO ₂	C ₁₆ H ₁₄ N ₄ O ₃	78	223-225	0.58
3k	<i>m</i> -NO ₂		71	245-247	0.67
3l	<i>p</i> -NO ₂		82	231-233	0.79
3m	<i>o</i> -CH ₃	C ₁₇ H ₁₇ N ₃ O	71	246-247	0.69
3n	<i>m</i> -CH ₃		70	245-248	0.68
3o	<i>p</i> -CH ₃		85	237-239	0.59
3p	<i>o</i> -OCH ₃	C ₁₇ H ₁₇ N ₃ O ₂	72	222-224	0.56
3q	<i>m</i> -OCH ₃		71	228-230	0.81
3r	<i>p</i> -OCH ₃		87	245-247	0.63

^aThe solvent system used was ethylacetate/hexane/methanol (4:6:2 v/v).

AutoGrid. The ligands used for docking were drawn using MDL ISIS Draw 2.5 and saved as a 'mol' file that was imported into ACD/ChemSketch to obtain a file format compatible for use in ArgusLab. After 3D optimization, molecule data were stored as a PDB file. All possible flexible torsions of the ligands were defined by AUTOTORS. Docking simulations were done with Auto Dock 4.2 using a Lamarckian genetic algorithm. Standard docking procedures were used for rigid proteins and flexible ligands with torsion angles that were identified. A grid consisting of 60, 60, and 60 points in the x, y, and z directions was created with its center at the catalytic site of the protein. Default settings were used for all other parameters. All calculations were performed on PCs running Windows XP. The resulting structures were analyzed using Auto Dock Tools.

2.4. MABA Assay protocol

The prepared compounds were tested for their *in vitro* activity against the H₃₇Ra strain of *M. tuberculosis*. An antimycobacterial bioassay was performed using the microplate Alamar Blue assay (MABA) (16). Briefly, representative colonies of the H₃₇Ra strain from the Lowenstein-Jensen (LJ) slope were suspended in 1 mL of distilled water and the turbidity was adjusted to match McFarland tube No.1 (10⁷ CFU/mL). This suspension was further diluted to 1:25 in 7H9 (Middlebrook 7H9 [Becton Dickinson] supplemented with 0.2% glycerol, 0.1% casitone, and 10% albumin-dextrose, pH 6.8) and used as the inoculum. One hundred μ L of the bacterial suspension was added to each well of a micro titer plate together with the synthesized compounds **3a-3r** in Middlebrook 7H9 medium to reach a final volume of 200 μ L. The final concentration of the test compounds, **3a-3r**, ranged from 31.25 μ g/mL to 0.97 μ g/mL. A growth control well and a sterile control well were also included on each plate. Plates were covered and sealed with Parafilm and incubated at 37°C. After incubation for about 7 days, 20 μ L of Alamar Blue dye were added to the wells. The plates were re-incubated overnight. A color change from blue to pink indicated bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of a compound that inhibited visible bacterial growth of *M. tuberculosis* and the MIC ranged from 0.0047-0.0095 (μ g/mL). The first line anti-TB drug INH served as the reference compound.

3. Results and Discussion

3.1. Chemistry

The series of heterocycles, 3-(((substituted phenyl) amino)methyl)-2-methylquinazolin-4(3H)-one **3a-3r**, was synthesized by the reaction of 2-methylquinazolin-4(3H)-one **2** with an appropriate solution of substituted aromatic amines as shown in the **Diagram**. The IR

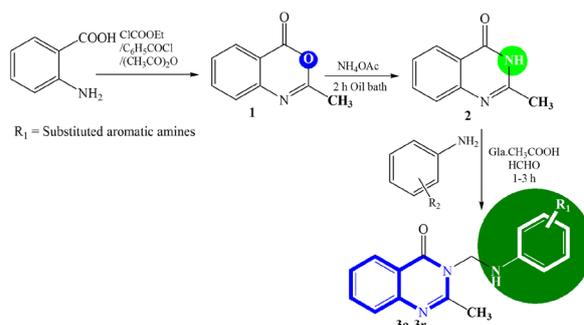


Diagram. Protocol for synthesis of the target compounds **3a-3r**

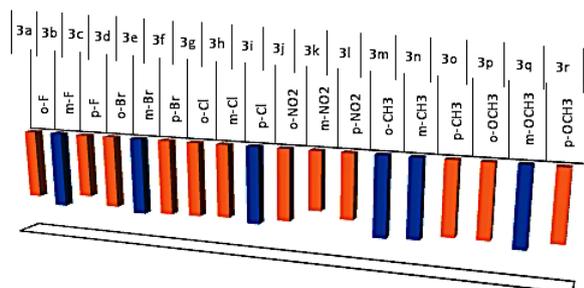
spectrum had a strong stretching band at 3,229 cm^{-1} due to the secondary amino group and no C-O-C stretching peak in the range of 1,300-1,300 cm^{-1} , confirming the formation of compound 2-methylquinazolin-4(3H)-one **2**. The IR spectra of the obtained compounds had stretching bands for C-F (1,009, 1,101, 1,031 cm^{-1}), C-Br (642, 598, 627 cm^{-1}), C-Cl (722, 714, 742 cm^{-1}), and N-O (1,502, 1,542, 1,498 cm^{-1}), confirming the identity of compounds **3a-3l**. Compounds **2** and **3a-3r** were formed based on the NH stretching peaks in the range of 3,300-3,500 cm^{-1} . According to ¹H NMR spectra, the obtained compounds produced a single peak at 9.31, 8.48, 9.24, 8.94, 9.14, 8.94, 8.99, 9.48, 9.14, 9.11, 8.41, 9.44, 8.93, 8.41, 9.34, 8.95, 8.49, 9.12, and 8.71 ppm due to the NH group, confirming the formation of the compounds in question (**3a-3r**). The conversion of 2-methylquinazolin-4(3H)-one **2** was apparent from a single peak at δ 4.55, 4.27, 4.47, 4.25, 4.12, 4.12, 4.35, 4.47, 4.42, 4.51, 4.24, 4.51, 4.23, 4.57, 4.56, 4.47, and 4.51 ppm due to the CH_2 proton, so ¹H NMR confirmed the formation of the compounds in question (**3a-3r**). In addition, mass spectra revealed the purity and molecular weight of those compounds. The above findings clearly indicated that the target compounds **3a-3r** were formed as indicated in the **Diagram** and had structures as were proposed.

3.2. In silico screening

Inhibition of DNA gyrase by 3-(((substituted phenyl) amino)methyl)-2-methyl quinazolin-4(3H)-one compounds **3a-3r** was indicated since they docked with the active site of DNA gyrase. Inhibition was tested experimentally, and the obtained K_i values correlate with that effect. The different scoring functions provide multiple approaches to evaluate ligand-receptor interactions and differing scores are expected to better aid in prioritization. Compounds **3m**, **3e**, **3b**, **3n**, **3q**, **3i**, and **3f** had the least free energy of binding. The free energy of binding and residues heavily interacting with compounds are shown in Table 2 and Figure 1. As an example, compounds **3m** (*o*-CH₃), **3e** (*m*-Br), and **3b** (*m*-F) had a free energy of binding of -1.19 kcal/mol and the ligand **3m** was found within the active site

Table 2. *In silico* and *in vitro* data on 3-(((substituted phenyl)amino)methyl)-2-methylquinazolin-4(3H)-one 3a-3r

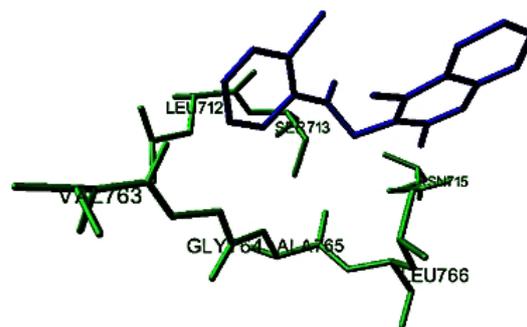
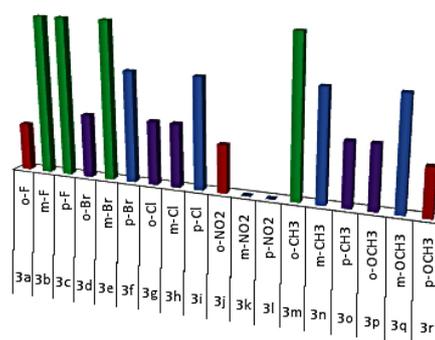
Compounds	Substitution	ΔG	Ki	MIC ($\mu\text{g/mL}$)
3a	<i>o</i> -F	-4.4	590	31.25
3b	<i>m</i> -F	-5.2	153.2	1.95
3c	<i>p</i> -F	-3.95	1.26	1.95
3d	<i>o</i> -Br	-4.75	330.8	15.62
3e	<i>m</i> -Br	-5.23	147.2	1.95
3f	<i>p</i> -Br	-5.0	215.7	7.81
3g	<i>o</i> -Cl	-4.91	250.38	15.62
3h	<i>m</i> -Cl	-4.76	321.95	15.62
3i	<i>p</i> -Cl	-5.16	165.46	7.81
3j	<i>o</i> -NO ₂	-4.55	458.83	31.25
3k	<i>m</i> -NO ₂	-3.45	2.95	Nil
3l	<i>p</i> -NO ₂	-3.92	1.34	Nil
3m	<i>o</i> -CH ₃	-5.38	114.23	1.95
3n	<i>m</i> -CH ₃	-5.2	153.41	7.81
3o	<i>p</i> -CH ₃	-4.63	406.25	15.62
3p	<i>o</i> -OCH ₃	-4.61	418.17	15.62
3q	<i>m</i> -OCH ₃	-5.17	163.18	7.81
3r	<i>p</i> -OCH ₃	-4.3	705.6	31.25
Rifampicin	-	-	-	0.0095

**Figure 1.** Graph of the binding energy (ΔG) of 3-(((substituted phenyl)amino)methyl)-2-methyl quinazolin-4(3H)-one 3a-3r.

residues ASN 715, ALA 765, LEU 712, LEU 766, VAL 763, TYR 237, and GLY 764 (Figure 2).

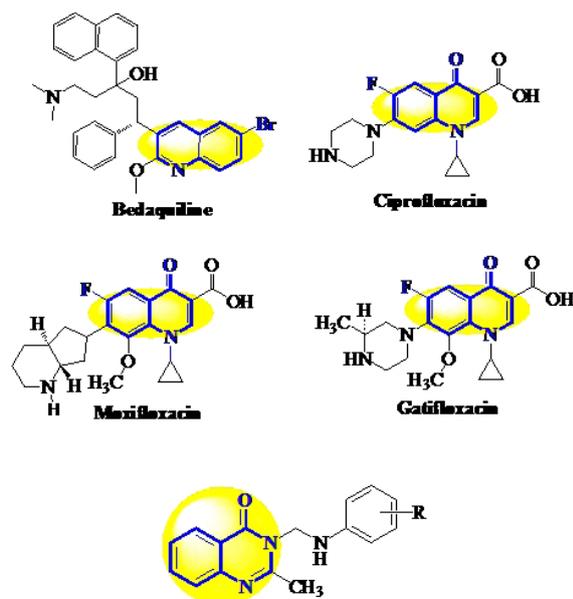
3.3. Screening of *in vitro* anti-TB activity

MABA was used to assay all 18 of the 3-(((substituted phenyl)amino)methyl)-2-methylquinazolin-4(3H)-one analogs, **3a-3r**, for their activity against *M. tuberculosis* strains at six different concentrations: 0.97, 1.95, 3.90, 7.81, 15.62, and 31.25 $\mu\text{g/mL}$. The effect of these synthetic compounds on the growth of the H₃₇Ra strain of *M. tuberculosis* was recorded after 7 days of incubation at 37°C. The observed anti-TB activity revealed that the compounds **3m** (*o*-CH₃), **3e** (*m*-Br), **3b** (*m*-F), and **3c** (*p*-F) were active against the H₃₇Ra strain at all concentrations except 0.97 $\mu\text{g/mL}$. According to MABA, compounds **3n** (*m*-CH₃), **3q** (*m*-OCH₃), **3i** (*p*-Cl), and **3f** (*p*-Br) were active against the H₃₇Ra strain at concentrations from 7.81-31.25 $\mu\text{g/mL}$, compounds **3g** (*o*-Cl), **3h** (*m*-Cl), **3d** (*o*-Br), **3o** (*p*-CH₃), and **3p** (*o*-OCH₃) were active at a concentration of 15.62 $\mu\text{g/mL}$, compounds, **3j** (*o*-NO₂), **3a** (*o*-F), and **3r** (*p*-OCH₃)

**Figure 2.** Interaction of the ligand 3-(((2-methylphenyl)amino)methyl)-2-methylquinazolin-4(3H)-one 3m receptor and DNA gyrase.**Figure 3.** Graph of the MIC of 3-(((substituted phenyl)amino)methyl)-2-methylquinazolin-4(3H)-one 3a-3r.

were active at a concentration of 31.25 $\mu\text{g/mL}$, and compounds **3l** (*p*-NO₂) and **3k** (*m*-NO₂) were inactive at all concentrations (Figure 3). An interesting finding is that compounds with *ortho*, *meta*, or *para* substitutions of electron-accepting groups (fluoro and bromo) and electron-donating groups (methyl and methoxy) had significant activity against the H₃₇Ra strain at all concentrations. The enhanced activity of these compounds means that they are highly potent against microbial strains and should display similar potency against *M. tuberculosis*.

There is an urgent need for the discovery and development of new anti-TB agents that target novel biochemical pathways and treat drug resistant forms of the disease. *M. tuberculosis* is unique in that it is surrounded by a thick and waxy cell wall, so efficient anti-TB drugs should have reasonable lipophilicity to penetrate the cell wall. The screened library contained a set of 3-(((substituted phenyl)amino)methyl)-2-methylquinazolin-4(3H)-one compounds, **3a-3r**, that are structurally related to the established drugs bedaquiline, moxifloxacin, gatifloxacin, and ciprofloxacin (Figure 4). The screened compounds consisted of 18 quinazoline analogs with various substituted groups, and most of these compounds were deemed against TB. According to an antimycobacterial bioassay, six of these compounds had significant anti-TB activity, six had modest anti-TB activity, five had slight anti-TB activity, and one was inactive. Table 2 shows the eighteen compounds, **3a-**



3-(((substituted phenyl)amino)methyl)-2-methylquinazolin-4(3H)-one

Figure 4. The structures of established drugs and the synthesized 3-(((substituted phenyl) amino) methyl)-2-methylquinazolin-4(3H)-one model compound with its pharmacophore features.

3r, with their level of activity against *M. tuberculosis*. Screening revealed that derivatives with *ortho*, *meta*, or *para* substitutions were very similar to the standard anti-TB drug rifampicin. In particular, compounds **3m** (*o*-CH₃), **3e** (*m*-Br), **3b** (*m*-F), and **3c** (*p*-F) had significant anti-TB activity and thus can be considered as potential sources of anti-TB agents. These compounds have all of the hallmarks of a new generation of anti-TB agents along with **3n** (*m*-CH₃), **3q** (*m*-OCH₃), **3i** (*p*-Cl), and **3f** (*p*-Br). Figures 1 and 3 also show a number of interesting aspects of quinazolinone that differ from the typical structure associated with different substitutions and anti-TB activity. Compounds with an *ortho*, *meta*, or *para* substitution had significant anti-TB activity, although there were inactive compounds with a *para* or *meta* NO₂ group. All of the tested compounds warrant a thorough discussion of their structure-activity relationship (SAR). There are interesting trends in terms of the activity that was noted. As an example, a quinazolinone with a substituted *meta* amine appears to have more potent activity than its close cousin with a substituted *ortho* or *para* amine.

4. Conclusion

In summary, a series of 3-(((substituted phenyl)amino) methyl)-2-methylquinazolin-4(3H)-one compounds, **3a-3r**, was synthesized and characterized with IR, ¹H NMR, mass spectroscopy, and elemental analyses. Activity was presumably the result of changing the substituents added to the quinazolinone core. The presence of *meta*-substituted fluoro, bromo, methyl, methoxy, or chloro groups on the aromatic amine ring increased the activity of compounds

in comparison to compounds with other substituents. A search of the literature for this specific scaffold yielded no previous reports in relation to TB. The current results shed light on how quinazolinone analogs with different substituted groups display different levels of activity. In light of the above findings regarding the effect of certain scaffolds on activity, the mode of action of the studied compounds should be determined and analogs should be prepared to develop a clear picture of their SAR. The analogs synthesized here will benefit future studies of quinazolinone by chemists and researchers.

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Appendix

Spectral data for the synthesized compounds

2-Methyl-4H-benzo[1,3]oxazin-4-one (1) IR (KBr) cm^{-1} : 3,000 (Ar-CH str), 2,942 (CH_3 -CH str), 1,715 (C=O), 1,636 (C=N), 1,057 (C-O-Cstr); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm: 2.32 (s, 3H, CH_3), 6.98-7.47 (m, 4H, Ar-H); MS (EI) m/z : 161 [M⁺]; Anal. Calcd for $\text{C}_9\text{H}_7\text{NO}_2$: C, 67.07; H, 4.38; N, 8.69. Found: C, 67.16; H, 4.40; N, 8.66.

2-methylquinazolin-4(3H)-one (2) IR (KBr) cm^{-1} : 3,229 (NH str), 3,012 (Ar-CH str), 2,882 (CH_3 -CH str), 1,710 (C=O), 1,629 (C=N); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm: 2.41 (s, 3H, CH_3), 6.92-7.40 (m, 4H, Ar-H), 9.31 (s, 1H, NH); MS (EI) m/z : 160 [M⁺]; Anal. Calcd for $\text{C}_9\text{H}_8\text{N}_2\text{O}$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.41; H, 5.09; N, 17.42.

3-(((2-fluorophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3a) IR (KBr) cm^{-1} : 3,385 (NH str), 3,035 (Ar-CH str), 2,970 (CH_3 -CH str), 1,700 (C=O), 1,009 (C-F); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm: 3.12 (s, 3H, CH_3), 4.55 (s, 2H, CH_2), 7.12-7.50 (m, 8H, Ar-H), 8.48 (s, 1H, NH); MS (EI) m/z : 283 [M⁺]; Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{FN}_3\text{O}$: C, 67.83; H, 4.98; N, 14.83. Found: C, 67.82; H, 4.91; N, 14.86.

3-(((3-fluorophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3b) IR (KBr) cm^{-1} : 3,397 (NH str), 3,013 (Ar-CH str), 2,921 (CH_3 -CH str), 1,711 (C=O), 1101 (C-F); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm: 2.92 (s, 3H, CH_3), 4.27 (s, 2H, CH_2), 7.52-8.18 (m, 8H, Ar-H), 9.24 (s, 1H, NH); MS (EI) m/z : 283 [M⁺]; Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{FN}_3\text{O}$: C, 67.83; H, 4.98; N, 14.83. Found: C, 67.86; H, 4.97; N, 14.87.

3-(((4-fluorophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3c) IR (KBr) cm^{-1} : 3,387

(NH str), 3,082 (Ar-CH str), 2,927 (CH_3 -CH str), 1,708 (C=O), 1,031 (C-F); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm: 2.44 (s, 3H, CH_3), 4.47 (s, 2H, CH_2), 7.12-8.10 (m, 8H, Ar-H), 8.94 (s, 1H, NH); MS (EI) m/z : 283 [M⁺]; Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{FN}_3\text{O}$: C, 67.83; H, 4.98; N, 14.83. Found: C, 67.87; H, 4.95; N, 14.81.

3-(((2-bromophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3d) IR (KBr) cm^{-1} : 3,344 (NH str), 3,020 (Ar-CH str), 2,842 (CH_3 -CH str), 1,702 (C=O), 642 (C-Br); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm: 3.17 (s, 3H, CH_3), 4.25 (s, 2H, CH_2), 6.92-7.86 (m, 8H, Ar-H), 9.14 (s, 1H, NH); MS (EI) m/z : 346 [M+2]; Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{BrN}_3\text{O}$: C, 55.83; H, 4.10; N, 12.21. Found: C, 55.85; H, 4.13; N, 12.24.

3-(((3-bromophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3e) IR (KBr) cm^{-1} : 3,417 (NH str), 3,003 (Ar-CH str), 2,901 (CH_3 -CH str), 1,706 (C=O), 598 (C-Br); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm: 3.42 (s, 3H, CH_3), 4.12 (s, 2H, CH_2), 7.12-7.28 (m, 8H, Ar-H), 8.94 (s, 1H, NH); MS (EI) m/z : 346 [M+2]; Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{BrN}_3\text{O}$: C, 55.83; H, 4.10; N, 12.21. Found: C, 55.84; H, 4.11; N, 12.21.

3-(((4-bromophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3f) IR (KBr) cm^{-1} : 3,407 (NH str), 3,009 (Ar-CH str), 2,900 (CH_3 -CH str), 1,712 (C=O), 627 (C-Br); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm: 3.02 (s, 3H, CH_3), 4.12 (s, 2H, CH_2), 7.12-7.90 (m, 8H, Ar-H), 8.99 (s, 1H, NH); MS (EI) m/z : 346 [M+2]; Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{BrN}_3\text{O}$: C, 55.83; H, 4.10; N, 12.21. Found: C, 55.78; H, 4.15; N, 12.28.

3-(((2-chlorophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3g) IR (KBr) cm^{-1} : 3,403 (NH str), 3,005 (Ar-CH str), 2,909 (CH_3 -CH str), 1,712 (C=O), 722 (C-Cl); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ ppm: 3.22 (s, 3H, CH_3), 4.35 (s, 2H, CH_2), 7.22-7.70 (m, 8H,

Ar-H), 9.48 (s, 1H, NH); MS (EI) m/z : 301 [M+2]; Anal. Calcd for $C_{16}H_{14}ClN_3O$: C, 64.11; H, 4.71; N, 14.02. Found: C, 64.15; H, 4.76; N, 14.00.

3-(((3-chlorophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3h) IR (KBr) cm^{-1} : 3,377 (NH str), 3,011 (Ar-CH str), 2,901 (CH₃-CH str), 1,701 (C=O), 714 (C-Cl); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 3.32 (s, 3H, CH₃), 4.47 (s, 2H, CH₂), 7.32-8.02 (m, 8H, Ar-H), 9.14 (s, 1H, NH); MS (EI) m/z : 301 [M+2]; Anal. Calcd for $C_{16}H_{14}ClN_3O$: C, 64.11; H, 4.71; N, 14.02. Found: C, 64.13; H, 4.73; N, 14.06.

3-(((4-chlorophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3i) IR (KBr) cm^{-1} : 3,417 (NH str), 3,012 (Ar-CH str), 2,907 (CH₃-CH str), 1,705 (C=O), 742 (C-Cl); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 2.47 (s, 3H, CH₃), 4.42 (s, 2H, CH₂), 7.22-8.40 (m, 8H, Ar-H), 9.11 (s, 1H, NH); MS (EI) m/z : 301 [M+2]; Anal. Calcd for $C_{16}H_{14}ClN_3O$: C, 64.11; H, 4.71; N, 14.02. Found: C, 64.09; H, 4.70; N, 14.03.

3-(((2-nitrophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3j) IR (KBr) cm^{-1} : 3,412 (NH str), 3,000 (Ar-CH str), 2,889 (CH₃-CH str), 1,707 (C=O), 1,502 (N-O); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 3.11 (s, 3H, CH₃), 4.51 (s, 2H, CH₂), 7.11-7.51 (m, 8H, Ar-H), 8.41 (s, 1H, NH); MS (EI) m/z : 310 [M+]; Anal. Calcd for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 4.55; N, 18.06. Found: C, 61.97; H, 4.58; N, 18.02.

3-(((3-nitrophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3k) IR (KBr) cm^{-1} : 3,497 (NH str), 3,014 (Ar-CH str), 2,941 (CH₃-CH str), 1,714 (C=O), 1,542 (N-O); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 2.94 (s, 3H, CH₃), 4.24 (s, 2H, CH₂), 7.54-8.14 (m, 8H, Ar-H), 9.44 (s, 1H, NH); MS (EI) m/z : 310 [M+]; Anal. Calcd for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 4.55; N, 18.06. Found: C, 62.00; H, 4.60; N, 18.10.

3-(((4-nitrophenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3l) IR (KBr) cm^{-1} : 3,337 (NH str), 3,032 (Ar-CH str), 2,937 (CH₃-CH str), 1,703 (C=O), 1,498 (N-O); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 2.34 (s, 3H, CH₃), 4.43 (s, 2H, CH₂), 7.13-8.30 (m, 8H, Ar-H), 8.93 (s, 1H, NH); MS (EI) m/z : 310 [M+]; Anal. Calcd for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 4.55; N, 18.06. Found: C, 61.92; H, 4.51; N, 18.07.

3-(((2-methylphenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3m) IR (KBr) cm^{-1} : 3,415

(NH str), 3,015 (Ar-CH str), 2,910 (CH₃-CH str), 1,710 (C=O); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 2.42 (s, 3H, CH₃), 3.12 (s, 3H, CH₃), 4.51 (s, 2H, CH₂), 7.11-7.51 (m, 8H, Ar-H), 8.41 (s, 1H, NH); MS (EI) m/z : 279 [M+]; Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.11; H, 6.14; N, 15.01.

3-(((3-methylphenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3n) IR (KBr) cm^{-1} : 3,337 (NH str), 3,033 (Ar-CH str), 2,931 (CH₃-CH str), 1,713 (C=O); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 2.32 (s, 3H, CH₃), 2.92 (s, 3H, CH₃), 4.23 (s, 2H, CH₂), 7.32-8.13 (m, 8H, Ar-H), 9.34 (s, 1H, NH); MS (EI) m/z : 279 [M+]; Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.15; H, 6.15; N, 15.07.

3-(((4-methylphenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3o) IR (KBr) cm^{-1} : 3,385 (NH str), 3,052 (Ar-CH str), 2,925 (CH₃-CH str), 1,705 (C=O); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 2.45 (s, 3H, CH₃), 3.45 (s, 3H, CH₃), 4.57 (s, 2H, CH₂), 7.52-8.15 (m, 8H, Ar-H), 8.95 (s, 1H, NH); MS (EI) m/z : 279 [M+]; Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.13; H, 6.17; N, 15.09.

3-(((2-methoxyphenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3p) IR (KBr) cm^{-1} : 3,375 (NH str), 3,003 (Ar-CH str), 2,911 (CH₃-CH str), 1,714 (C=O); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 2.45 (s, 3H, CH₃), 3.17 (s, 3H, OCH₃), 4.56 (s, 2H, CH₂), 7.10-7.74 (m, 8H, Ar-H), 8.49 (s, 1H, NH); MS (EI) m/z : 295 [M+]; Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.19; H, 5.81; N, 14.27.

3-(((3-methoxyphenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3q) IR (KBr) cm^{-1} : 3,431 (NH str), 3,009 (Ar-CH str), 2,907 (CH₃-CH str), 1,701 (C=O); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 2.37 (s, 3H, CH₃), 3.12 (s, 3H, OCH₃), 4.47 (s, 2H, CH₂), 7.12-7.73 (m, 8H, Ar-H), 9.12 (s, 1H, NH); MS (EI) m/z : 295 [M+]; Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.11; H, 5.83; N, 14.21.

3-(((4-methoxyphenyl)amino)methyl)-2-methylquinazolin-4(3H)-one (3r) IR (KBr) cm^{-1} : 3,415 (NH str), 3,031 (Ar-CH str), 2,911 (CH₃-CH str), 1,701 (C=O); ¹H-NMR (300 MHz, CDCl₃): δ ppm: 2.31 (s, 3H, CH₃), 2.95 (s, 3H, OCH₃), 4.51 (s, 2H, CH₂), 7.44-7.97 (m, 8H, Ar-H), 8.71 (s, 1H, NH); MS (EI) m/z : 295 [M+]; Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.19; H, 5.81; N, 14.25.