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Synthesis and Evaluation of In-Vitro Antimicrobial Activity of Some Novel 2-Styryl-3-Substituted-(3*H*) –Quinazolin–4–one derivatives

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Original Research Article

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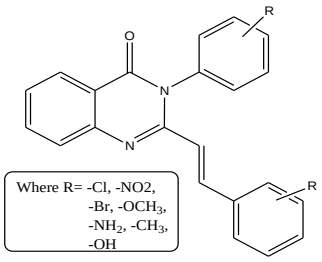
Abstract: (3*H*)–quinazolin–4–ones were reported for enhanced antibacterial, antiviral, antifungal and antimalarial activities with aryl and styryl substituents at position two and three respectively. In the present work, synthesis of some newly substituted (3*H*)–quinazolin–4–ones starting from 2–aminobenzoic acid and acetic anhydride producing 2–methyl–benzoxazin–4–one **(**Step–I) has been undertaken. Replacement of ring oxygen from benzoxazin–4–one (step–II) with nitrogen atom of 4–chloro aniline produced first series of title compounds (QIa-QIk). In addition, 2-methyl group of benzoxazin–4–one was extended further as ethenyl (–CH=CH–) linkage joining to various substituted aldehydes (Step–III). It has produced sufficient quantity of 2–styryl–3–aryl–(3H)–quinazolin–4–ones (QIa–QIk, Scheme I). In obtaining second series compounds (QIl–QIt), identical approach was followed with replacement of benzoxazin-4-one ring oxygen with nitrogen atom of various substituted amines (Step–II).There is extension of methyl group of benzoxazin–4–one as ethenyl group (–CH=CH–) joining 2–chloro benzaldehyde. It has produced sufficient quantity of 2-[2-(2-Chloro-phenyl)-vinyl]-3-aryl–(3H)–quinazolin–4–one (QIl–QIt, Scheme II). Structures of synthesized compounds were established by elemental analysis (C, H, N, O and X=halogen), FT–IR, ^{1}H NMR, ^{13}C NMR and HR–MS. In-vitro **d**isk diffusion assay was performed using Gram–positive bacteria *Bacillus subtilis* (NCIM2711), *Staphylococcus aureus* (NCIM2079), Gram–negative bacteria *Kleibsella pneumonia* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC27853) and fungi *Candida albicans*, *Aspergillus niger*. Ciprofloxacin and Griseofulvin were the positive control standards for bacteria and fungi respectively. Excellent antimicrobial activity (MIC=9.07–10.54 µM x 10^{-3}) was exhibited by compounds as (QId), (QIf), (QIg), (QIi), (QIj), (QIk), (QIl), (QIm), (QIn), (QIq), (QIr) and (QIs). Present study of (3*H*)–quinazoline–4–ones will serve as an important path in the development and discovery of newer antimicrobial agents.
Keywords: (3*H*)–quinazoline–4–one, antibacterial, antifungal, disc diffusion assay, MIC.

INTRODUCTION

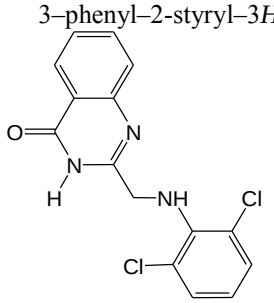
Among nitrogen containing heterocyclic compounds quinazolinones have played an important role and explored to develop pharmacophore molecules having biological importance [1-3]. Quinazolinones are reported to exhibit therapeutic activities including antibacterial [4, 5], antiviral [6], antifungal [7, 8], antimalarial [9]. Although rarely described, synthetic quinazolinone ring is a part of several antibiotics, known to inhibit growth of Gram–positive bacteria. Some quinazolin–4–one scaffold containing diverse molecules were reported to exhibit good antimicrobial activity (Figure-1). Novel quinazolinone–thiazolidine–quinoline compounds possessing methyl group at para position and hydroxy group at ortho position were reported to exhibit remarkable antibacterial and antifungal activity [10]. Biological importance of 3–amine substituted quinazolin–4(3*H*)–One derivatives producing antibacterial activity and 6–substituted indolo[1,2–c]quinazolines contributing antimicrobial activity was described previously [11, 12].

Quinazolin–4–one derivatives substituted at position two and three with electron rich substituents, hydroxyl, amines, substituted amines, nitro group and halogens play an important role to produce different biological activities [13]. Substitutions at these positions are reported to enhance antibacterial and antitubercular activities of (3*H*)–quinazolin–4–ones [14-17]. (3*H*)–quinazolin–4–one derivatives are reported for significant antimicrobial activity against different species of Gram–positive bacteria, Gram–negative bacteria and pathogenic fungi [18-20]. Quinazolinone ring system is found in many biologically active natural products as Asperlicin C, Sclerotigenin,

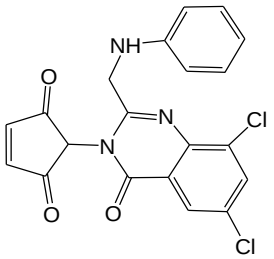
Circumdatin F, Benzomalvin A [21-23]. Although vast number of antimicrobial agents is available in the market, the search for new molecules with minimal side–effects and broad spectrum of activity are preferred more. The epidemic of resistant pathogens has spurred the renewed concern in finding novel antimicrobial agents. Since the quinazolinone scaffold seems to be a possible pharmacophore in various pharmacologically active agents, in this endeavour our research group has decided for efficient synthesis of (3*H*)–quinazoline–4–one analogues (QIa–QIt) and their evaluation against pathogenic micro–organisms. Quinazoline nucleus containing pyrimidine ring moiety as a part of its structure, fused further at position two and three with substituted pharmacophore moieties. Substitution of aldehyde and ketone moieties at their respective positions may able to elicit and enhance antimicrobial activity of scaffold compound under synthesis. Influence of electron–donating substituents such as methoxyl and amino groups on antimicrobial activity was studied further. Present study of (3*H*)–quinazoline–4–one derivatives could serve as an important path for discovery of newer chemical entities to treat clinically pathogenic microbial infections.



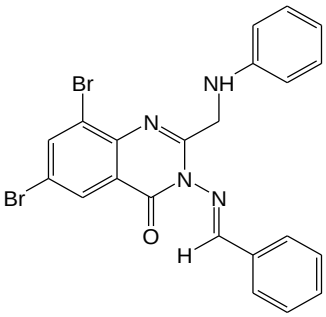
3–phenyl–2-styryl–3*H*–quinazolin–4–one skeleton of target compounds



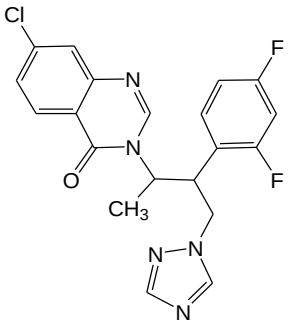
2-[(2,6-Dichloro-phenylamino)-methyl]-3*H*-quinazolin-4-one



2-(6,8-Dichloro-4-oxo-2-phenylaminomethyl-4*H*-quinazolin-3-yl)-cyclopent-4-ene-1,3-dione



3-(Benzylidene-amino)-6,8-dibromo-2-phenylaminomethyl-3*H*-quinazolin-4-one



7-Chloro-3-[2-(2,4-difluoro-phenyl)-1-methyl-3-[1,2,4] triazol-1-yl-propyl]-3*H*-quinazolin-4-one

Fig-1: Examples of Quinazoline–4–ones reported as potential antimicrobial agents

MATERIALS AND METHODS

Experimental details

All reactions except those in aqueous media were carried out by standard techniques with devoid of moisture. Melting points were checked with help of Fischer–Jones melting point apparatus and were reported uncorrected. Elemental analysis (% C, H, N, O and X=halogen) was carried out using a Rapid Microanalyser at University of Pune, (MS), India. TLC analysis was carried out on silica gel–protected Aluminum sheets (Type 60 F 81.174, Merck) and spots were detected under UV–Lamp at λ 364 nm and were used for purity checking and reaction monitoring. Column chromatography on silica gel (Merck, 70–230 and 230–400 mesh ASTH for flash chromatography) was applied when necessary to isolate and purify the reaction products. FT–IR spectra of all synthesized compounds have been recorded on a JASCO 401 FT–IR spectrometer in KBr background. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ as a solvent by BRUKER AVANCE II FT–NMR spectrometer (400 MHz) using TMS (Tetra Methyl Silane) as internal standard (chemical shifts in δ, ppm), s=singlet, d=doublet, m=multiple, bs=broad singlet. The relative integrals of peak

areas agreed with those expected for the assigned structures. Mass spectra were recorded on WATERS Q–TOF Micro mass (LC–MS), performed at SAIF, Punjab University, Chandigarh, India.

Chemistry

The purity of all the synthesized compounds was checked by TLC method. The structures of compounds were established by spectroscopic methods listed above. The infrared spectra of all compounds exhibited intense absorption bands in the range of 1680–1623 cm^{-1} and 3010–2910 cm^{-1} as characteristic of ethenylic (–CH=CH– stretch) and (=C–H stretch) respectively. In the ^{1}H NMR spectra of all compounds presence of single integration signal at 5.9–4.6 ppm derived from one proton, indicating ethenylic (–C=C–H) group. Intense bands in FT–IR spectra at 1775–1725 cm^{-1} originating from the vibrations of 4–one (C=O stretch) of quinazoline nucleus indicate presence of carbonyl group in the quinazoline nucleus. FT–IR spectra with strong absorption at 1690–1650 cm^{-1} is indicating presence of aromatic (C=N stretch) in the quinazoline ring. Intense FT–IR absorption bands at 1650-1540 cm^{-1} and 3100-3000 cm^{-1} designate presence of aromatic (C=C stretch) and (C–H stretch) vibrations respectively. In the ^{1}H NMR spectra of all compounds presence of single integration signal at 8.5–6.0 ppm derived from (C–H) aromatic proton. Additionally, FT–IR spectra with vibrations in the range of 850–700 cm^{-1} indicate presence of (C–Cl stretch) in the structure of all compounds. These are the spectroscopic characteristics of synthesized quinazolin–4–one compounds showing common absorption bands in the FT–IR and ^{1}H NMR spectra.

FT–IR spectra of compounds QIc, QIj, QIk, QIo and QIp observed with the common absorption bands in the range of 1250–1189 cm^{-1} arrived because of (C–O stretch) of $–OCH_3$ group. Whereas, ^{1}H NMR absorption at 4.0–3.3 ppm derived from three protons with single integration signal designate presence of $–OCH_3$ group in the structure of similar compounds. In the FT–IR absorption spectra of compounds QId, QIq, QIr and QIt presence of symmetric stretching bands of N–O in the range of 1475–1360 cm^{-1} confirm availability of nitro group within molecule.

Compounds as QIc, QIe, QIg and QIh shows FT–IR stretching vibrations in the range of 3650–3550 cm^{-1} indicates presence of (O–H) in hydroxyl group of the said molecule. However, intensive absorption in the ^{1}H NMR spectra of QIg and QIh at 4.0–3.4 indicates aliphatic O–H (QIg, QIh) and 8.7–4.2 ppm (QIc, QIe) designate aromatic hydroxyl group. Presence of dimethylamine group in the compound QIb is indicated by FT–IR spectra of molecule with strong absorption bands in the range of 3450–3300 cm^{-1} because of N–H stretching vibrations however, intense aliphatic C–H stretching vibrations of methyl group at 3100–2950 cm^{-1}. In the ^{1}H NMR spectra of same compound (QIb) single integration band at 1–5 ppm derived from one proton of N–H group and another intense band at 0.9–1.0 derived from three protons of methyl group.

FT–IR absorption spectrum of compound QIm with intense absorption at 710–691 cm^{-1} indicates presence of C–Br group in the structure. FT–IR absorption spectra of compound QIn shows intense absorption in the range of 3000–2950 cm^{-1} as C–H stretching vibrations of methyl group. In the ^{1}H NMR spectra of same compound (QIb) presence of single integration band at 0.9–1.1 ppm derived from three protons of methyl group.

General procedure for synthesis of 3–phenyl–2–styryl–(3*H*)–quinazolin–4–ones [24, 25] (**Scheme 1, QIa–QIk**), **(Scheme 2, QIl–QIt)**

Synthesis of 2–methyl–1,3–benzoxazin–4–one (Step–I)

A reaction mixture of 5g of anthranilic acid (0.036 mol) and 7.4g of acetic anhydride (0.72 mol) was poured in round bottom flask. The reaction mixture was heated gently initially and further continued to heat under reflux for 1 hour. Upon completion, excess of acetic anhydride was distilled off under reduced pressure. Remaining concentrate from the distillating flask was poured on to crushed ice with constant stirring. Crude product obtained in the form of solid mass was dried in oven and further purified by recrystallization using (8:2) chloroform and benzene as solvent.

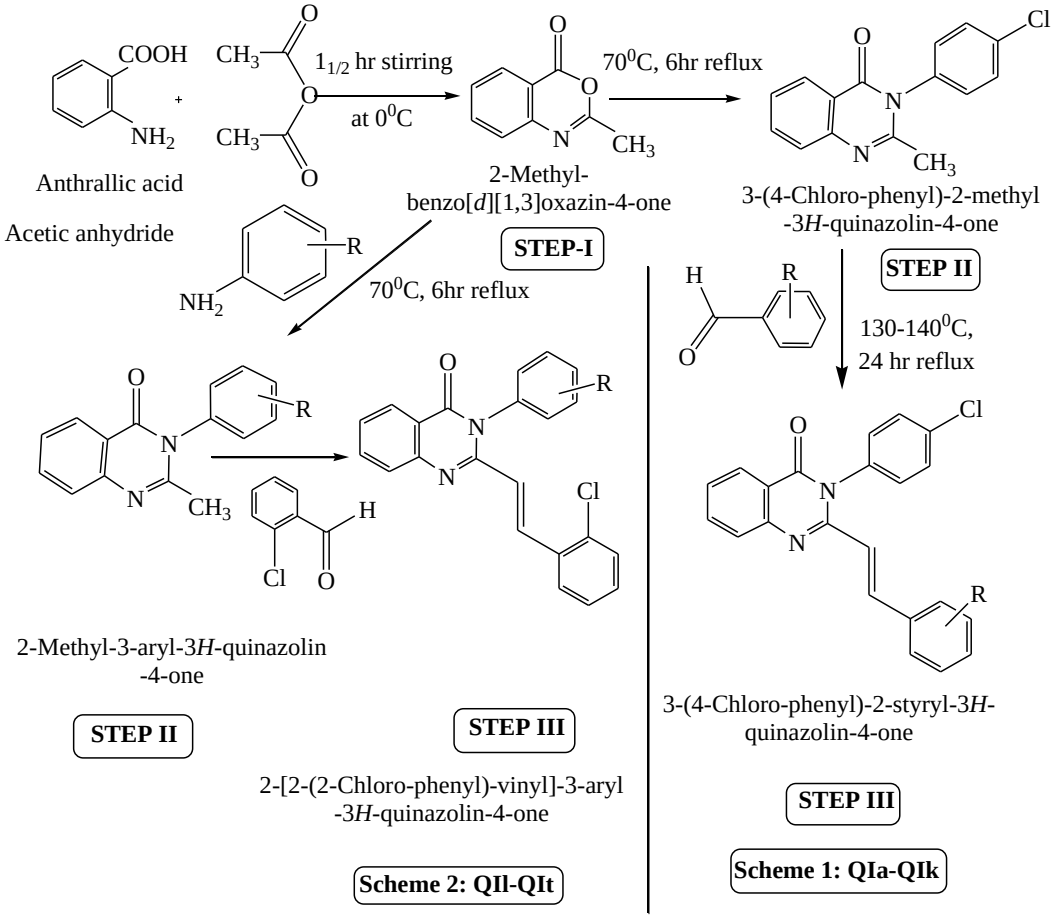
Synthesis of 2–methyl–3–aryl (3*H*)–quinazoline–4–one (Step–II) [26]

An equimolar (0.01mol) quantity of 2–methyl–1,3–benzoxazin–4–one (Step–I) and 4–chloro–aniline (Scheme 1, QIa–QIk) were mixed and heated to reflux for 5–6 hours with glacial acetic acid. Whereas different substituted aryl amines (Scheme 2, QIl–QIt) along with 2–methyl–1,3–benzoxazin–4–one were refluxed for 6 hours with glacial acetic acid. After completion, the reaction mixture was cooled to room temperature and poured in to crushed ice with constant stirring. The crude solid mass obtained was purified by recrystallization using chloroform.

Synthesis of 2, 3–disubtituted (3*H*)–quinqzolin–4–one (Step III) [27-29]

A reaction mixture with appropriate quantity of 2–methyl–3–aryl–(3*H*)–quinazoline–4–one (step–II) (0.01mol) and various substituted aldehydes (0.01mol) (Scheme 1, QIa–QIk) in 50 mL glacial acetic acid were stirred for 10 minutes at room temperature. The reaction mixture was heated to reflux continuously for 24 hours. Whereas reaction of 2–methyl–3–aryl–(3*H*)–quinazoline–4–one (Step–II, 0.01mol) with equimolar 2–chloro-benzaldehyde (Scheme 2,

QIl–QIt) in glacial acetic acid was carried out by reflux for 24 hrs. After reflux, reaction mixture was cooled to room temperature and poured slowly in to ice cold water with constant stirring. The crude precipitate obtained was filtered and dried in oven. Recrystallisation was performed in benzene to obtain purified compounds.



Synthesis of (3*H*)–quinazolin–4–ones from 2–amino benzoic acid (Scheme 1 and 2)

Biological Activity

Antibacterial Screening

Synthesized compounds were evaluated for antibacterial activity against Gram–positive bacteria [30] *Bacillus subtilis* (NCIM2711), *Staphylococcus aureus* (NCIM2079), and Gram–negative bacteria *Kleibsella pneumoniae* (ATCC 4352) and *Pseudomonas aeruginosa* (ATCC27853). Antibacterial activity was measured as per National Committee for Clinical Laboratory Standards (NCCLS) protocol by Mueller Hinton broth (Becton–Dickinson, USA) [31]. Standard strains were procured from Dr. D. Y. Patil Hospital and Medical Research Centre, Pimpri, Pune–411018 (MS). Compounds were primarily screened for their antibacterial activity in six sets against micro–organisms of different concentrations of 200, 100, and 50µg/mL. Those compounds found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.25 and 3.12 µg/mL concentrations for secondary screening. Inoculums size for test strain was adjusted by comparing the turbidity (turbidimetric method) up to 10^6 CFU/mL (Colony Forming Unit per milliliter). Synthesized compounds were diluted to 1,000 µg/mL concentration in respective solvents, to prepare stock solution. Control tube containing no antibiotic was immediately sub–cultured (before inoculation) by spreading a loopful of culture evenly over a quarter part of medium in a plate suitable for the growth of test organism. The tubes with bacterial culture were shifted for incubation at 37 ^{0}C for 24 hrs. Suspensions of 10µM were further inoculated on appropriate media, and growth was noted after 24 and 48 hours.

The lowest concentration of test compound, observed with no microbial growth after spot subculture was considered as MIC for each test compound. The highest dilution, that is with lowest test compound concentration preventing appearance of turbidity was measured as MIC. It is nothing but amount of growth from the control tube before incubation (which represents the original inoculum) was compared. The test mixture should contain 10^6 CFU/mL of culture organisms, 2% DMSO where, sterile distilled water was used as a negative control. A set of tubes containing only seeded broth and solvent control were maintained under identical conditions to make sure about diluting solvent had no influence on microbial growth. The result of this was significantly affected by the size of inoculum. In the present study

Ciprofloxacin (1 U strength) was considered as positive control for evaluating antibacterial activity and it was found with MIC=18.08 µM x 10^{-3} against *Bacillus subtilis*, *Staphylococcus aureus*, and *Kleibsella pneumoniae***,** *Pseudomonas aeruginosa.* Mueller Hinton broth has been used as a medium for bacterial growth allowed for aerobic incubation at 37 ^{o}C for 24 and 48 hours.

Antifungal Screening

The synthesized compounds were also tested for antifungal activity against selected fungal strains. During primary screening evaluation of test compounds in six sets against *Candida albicans*, *Aspergillus niger* at various concentrations of 100, 50, 25, 12.5, 6.25 and 3.12 µg /mL. Separate recording of results were done for primary and secondary screening as described previously. Test compounds were diluted with appropriate solvent to 1,000 µg /mL concentration, as a stock solution. Evaluated test compounds those found active in this primary screening were further used in second set of dilution against all fungi. Griseofulvin (1 U strength) was used as a positive control for antifungal activity, with observed MIC=19.25 µM x 10^{-3}. Dimethyl sulphoxide (DMSO, 2%) and sterile distilled water were used as negative control. Sabouraud's dextrose broth medium used for fungal growth was allowed for aerobic incubation at 28 ^{o}C for 48 hours. Summary of biological activity of evaluated compounds was summarized in Table-1.

Physical constants and characterization of 3-(4-Chloro-phenyl)-2-styryl-(3H)-quinazolin-4-one (QIa-QIk)

3-(4-chlorophenyl)-2-[(E)-2-(4-hydroxyphenyl)ethenyl]quinazolin-4(3H)-one (QIa): White crystalline powder, yield 89 %; mp 88-93 ^{o}C; IR (KBr) υ_{max}/cm^{-1} 3650 (O-H, aromatic), 3117.0 (C-H, aromatic), 3010.0 (=C-H, ethenylic), 1650.4 (C=C, ethenylic), 1775 (C=O), 1690 (C=N, aromatic), 1580 (C=C, aromatic), 1200 (C-N, aromatic), 1275 (C-O, aromatic), 875 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): δ= 7.49 (4H, d, *J*=0.5Hz, aromatic), 7.81 (4H, d, *J*=0.684 Hz, aromatic), 5.53 (2H, s, C<u>H</u>=C<u>H</u>, ethenylic), 8.45 (1H, s, O<u>H</u>); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 112 (C18, CH), 136 (C19, CH), 156.5 (C23); LCMS (m/z): 374.81 $(M)^{+}$; Anal. Calcd for $C_{22}H_{15}ClN_2O_2$: C, 70.43; H, 4; N, 7.47; O, 8.53; Cl, 9.47. Found: C, 70.41; H, 4.12; N, 7.39; O, 8.42; Cl, 9.39.

3-(4-chlorophenyl)-2-{(E)-2-[4-(dimethylamino)phenyl]ethenyl}quinazolin-4(3H)-one (QIb:) This compound was prepared by the method described above. It was obtained as faint yellow crystalline powder, yield 73 %; mp 115-119 ^{o}C; IR (KBr) υ_{max}/cm^{-1} 3400 (N-H), 3123.0 (C-H-aromatic), 2980.0 (=C-H, ethenylic), 2975 (C-H, CH_3), 1650 (C=C, ethenylic), 1780 (C=O), 1690 (C=N, aromatic), 1576 (C=C, aromatic), 1250 (C-N, aliphatic), 1265 (C-N, aromatic), 875 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): δ= 7.67 (4H, d, *J*=0.45Hz, aromatic), 7.39 (4H, d, *J*=0.68 Hz, aromatic), 6.76 (4H, d, *J*=1.11Hz, aromatic), 5.53 (2H, s, C<u>H</u>=C<u>H</u>, ethenylic), 3.81 (6H, s, $(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 43.6 (-$\underline{C}H_3$), 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 129.1 (C14), 112 (C18, <u>C</u>H), 136 (C19, <u>C</u>H), 143.7 (C23); LCMS (m/z): 401.88 $(M)^{+}$; Anal. Calcd for $C_{24}H_{20}ClN_3O$: C, 71.66; H, 4.97; N, 10.45; O, 3.98; Cl, 8.83. Found: C, 71.62; H, 4.93; N, 10.42; O, 3.93; Cl, 8.56.

3-(4-chlorocyclohexa-1,3-dien-5-yn-1-yl)-2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]quinazolin-4(3H)-one (QIc): This compound was prepared by the method described above. It was obtained as white crystalline powder, yield: 79%; mp 89-95 ^{o}C; IR (KBr) υ_{max}/cm^{-1}: 3650 (O-H, aromatic), 3120.0 (C-H, aromatic), 3010.0 (=C-H, ethenylic), 2950 (C-H, aliphatic), 1635 (C=C, ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1585 (C=C, aromatic), 1350 (C-N, aliphatic), 1275 (C-N, aromatic), 1189 (C-O, aliphatic -OCH_3), 880 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): δ=8.45 (1H, s, O<u>H</u>), 7.95 (4H, d, *J*=0.9Hz, aromatic), 7.55 (4H, d, *J*=0.63Hz, aromatic), 7.25 (4H, d, *J*=0.93Hz, aromatic), 5.63 (2H, s, C<u>H</u>=C<u>H</u>, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ=56.3 (-$\underline{C}H_3$), 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 129.4 (C14), 112 (C18, CH), 136 (C19, CH), 149.1 (C22), 142.1 (C23); LCMS (m/z): 404.32 $(M)^{+}$; Anal. Calcd for $C_{23}H_{17}ClN_2O_3$: C, 68.17; H, 4.19; N, 6.91; O, 11.85; Cl, 8.76; Found: C, 68.21; H, 4.20; N, 6.84; O, 11.78; Cl, 8.75.

3-(4-chlorophenyl)-2-[(E)-2-(2-nitrophenyl)ethenyl]quinazolin-4(3H)-one (QId): This compound was prepared by the method described above. It was obtained as orange crystalline powder, yield 89%; mp 105-109 ^{o}C; IR (KBr) υ_{max}/cm^{-1}: 3093.70 (C-H, aromatic), 2995.0 (=C-H, ethenylic), 2900 (C-H, aliphatic), 1672.50 (C=C, ethenylic), 1769.5 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1560.3 (N=O), 1350 (C-C, aliphatic), 1275 (C-N, aromatic), 875 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): δ= 7.87 (4H, d, *J*=0.85Hz, aromatic), 7.47 (4H, d, *J*=0.63, aromatic), 6.64 (4H, d, *J*=0.93, aromatic), 5.30 (2H, s, CH=CH, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 129.4 (C14), 112 (C18, CH), 136 (C19, CH), 149.1 (C22), 142.1 (C23); LCMS (m/z): 403.83 $(M)^{+}$; Anal. Calcd for $C_{22}H_{14}ClN_3O_3$: C, 65.37; H, 3.46; N, 7.16; O, 12.28; Cl, 9.08; Found: C, 65.32; H, 3.44; N, 7.10; O, 12.29; Cl, 9.06.

3-(4-chlorophenyl)-2-[(E)-2-(3,4-dihydroxyphenyl)ethenyl]quinazolin-4(3H)-one (QIe): This compound was prepared by the method described above. It was obtained as white crystalline powder, yield: 78%; mp 140-143 ^{o}C; IR (KBr) υ_{max}/cm^{-1}: 3550 (O-H, aromatic), 3186.0 (C-H, aromatic), 2900 (C-H, aliphatic), 3081 (C=C, ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1590 (C=C, aromatic), 1345 (C-C, aliphatic), 1280 (C-N, aromatic), 1189 (C-O, aliphatic), 875 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): δ= 7.75 (4H, d, *J*=0.93Hz, aromatic), 7.47 (4H, d, *J*=0.63Hz, aromatic), 6.69

(4H, d, *J*=0.44Hz, aromatic), 5.60 (2H, s, CH=CH, ethenylic), 8.6 (1H, s, OH); ^{13}C NMR ($CDCl_3$, 400 MHz,): *δ*= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 129.4 (C14), 112 (C18, CH), 136 (C19, CH), 144.4 (C22), 143.7 (C23), 117 (C24), 120 (C81.17); LCMS (m/z): 390.78 $(M)^+$; Anal. Calcd for $C_{22}H_{15}ClN_2O_3$: C, 67.55; H, 3.83; N, 7.16; O, 12.28; Cl, 9.08; Found: C, 67.52, H, 3.87, N, 7.12; O, 12.22; Cl, 9.06.

3-(4-chlorophenyl)-2-[(E)-2-(2-chlorophenyl)ethenyl]quinazolin-4(3H)-one (QIf): This compound was prepared by the method described above. It was obtained as grey white crystalline powder, yield: 86%; mp 116-119 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3177.12 (C-H, aromatic), 3010.1 (C-H, ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1280 (C-N, aromatic), 880 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): *δ*= 7.49 (4H, d, *J*=0.63Hz, aromatic), 7.81 (4H, d, *J*=0.91Hz, aromatic), 6.69 (4H, d, *J*=0.12Hz, aromatic), 5.54 (2H, s, CH=CH, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): *δ*= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 129.4 (C14), 112 (C18, CH), 136 (C19, CH), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 129.5 (C24), 127.6 (C81.17); LCMS (m/z): 392.26 $(M)^+$; Anal. Calcd for $C_{22}H_{14}Cl_2N_2O$: C, 67.13; H, 3.55; N, 7.11; O, 4.06; Cl, 18.05; Found: C, 67.11, H, 3.53; N, 7.14; O, 4.05; Cl, 18.07.

3-(4-chlorophenyl)-2-[(1E)-3,4,5,6-tetrahydroxyhex-1-en-1-yl]quinazolin-4(3H)-one (QIg): This compound was prepared by the method described above. It was obtained as faint brown crystalline powder, yield: 89%; mp 99-104 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3587 (O-H), 3145 (C-H, aromatic), 2967 (=C-H, ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1280 (C-N, aromatic), 1100 (C-O aliphatic), 889 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): *δ*= 8.56 (4H, m, *J*=1.1Hz, O-H aliphatic), 7.68 (4H, d, *J*=0.63Hz, aromatic), 7.39 (4H, d, *J*=1.0Hz, aromatic), 6.60 (4H, d, *J*=0.9Hz, aromatic), 5.39 (2H, s, CH=CH, ethenylic), 3.78 (3H, s, CH_3), 8.56 (1H, s, OH); ^{13}C NMR ($CDCl_3$, 400 MHz,): *δ*= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 129.4 (C14), 114 (C18, CH), 141 (C19, CH), 73.8(C20), 78.2 (C23), 64.8 (C27); LCMS (m/z): 401.82 $(M)^+$; Anal. Calcd for $C_{20}H_{19}ClN_2O_5$: C, 59.57; H, 4.71; N, 6.95; O, 19.85; Cl, 8.81; Found: C, 59.56; H, 4.73; N, 6.94; O, 19.82; Cl, 8.79.

3-(4-chlorophenyl)-2-[(1E)-3,4,5,6-tetrahydroxyhex-1-en-1-yl]quinazolin-4(3H)-one (QIh): This compound was prepared by the method described above. It was obtained as brown crystalline powder, yield: 79%; mp 120-123 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3587 (O-H), 3145 (C-H, aromatic), 2967 (=C-H, ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1280 (C-N, aromatic), 1100 (C-O, aliphatic), 889 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): *δ*= 7.68 (4H, d, *J*=1.1Hz, aromatic), 7.39 (4H, d, *J*=0.46Hz, aromatic), 6.60 (4H, d, *J*=0.54Hz, aromatic), 5.39 (2H, s, CH=CH, ethenylic), 3.78 (3H, s, CH_3), 8.7 (1H, s, OH); ^{13}C NMR ($CDCl_3$, 400 MHz,): *δ*= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 129.4 (C14), 114 (C18, CH), 141 (C19, CH), 74.1(C20), 76 (C23), 72.4 (C81.17), 74.3 (C27), 64.7 (C29); LCMS (m/z): 431.82 $(M)^+$; Anal. Calcd for $C_{20}H_{19}ClN_2O_5$: C, 59.57; H, 4.71; N, 6.95; O, 19.85; Cl, 8.81; Found: C, 59.56; H, 4.73; N, 6.94; O, 19.82; Cl, 8.79.

3-(4-chlorophenyl)-2-[(E)-2-(furan-2-yl)ethenyl]quinazolin-4(3H)-one (QIi): This compound was prepared by the method described above. It was obtained as faint yellow crystalline powder, yield: 62%; mp 145-150 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3189.2 (C-H, aromatic), 2945 (=C-H, ethenylic), 1781.17 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1280 (C-N, aromatic), 1289 (C-O, furyl), 885 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): *δ*= 7.35 (4H, d, *J*=0.31Hz, aromatic), 7.43 (4H, t, *J*=0.44Hz, aromatic), 5.6 (2H, s, CH=CH, ethenylic), 6.3 (3H, d, *J*=0.11Hz, furyl); ^{13}C NMR ($CDCl_3$, 400 MHz,): *δ*= 145 (C2), 112.7 (C3), 111.8 (C4), 155.3 (C5), 136 (C6), 118 (C7), 164 (C8), 165 (C10), 127.4 (C11), 147.8 (C12), 128.6 (C14), 127.1(C15), 133.2 (C16), 122.1 (C17), 136.3 (C18), 121.8 (C19), 129.1(C20), 129.4 (C21), 129.1 (C22), 121.8 (C23); LCMS (m/z): 347.78 $(M)^+$; Anal. Calcd for $C_{20}H_{13}ClN_2O_2$: C, 75.69; H, 3.72; N, 8.02; O, 9.17; Cl, 10.17; Found: C, 75.65; H, 3.70; N, 8.05; O, 9.14; Cl, 10.13.

3-(4-chlorophenyl)-2-[(E)-2-(4-methoxyphenyl)ethenyl]quinazolin-4(3H)-one (QIj): This compound was prepared by the method described above. It was obtained as light brown crystalline powder, yield: 69%; mp 184-189 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3178.23 (C-H, aromatic), 2950 (C-H, CH_3), 2973 (=C-H, ethenylic), 3123 (C=C, ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1189 (C-O, aliphatic –OCH_3), 879 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): *δ*= 7.67 (4H, d, *J*=0.86Hz, aromatic), 7.39 (4H, d, *J*=0.35Hz, aromatic), 6.60 (4H, d, *J*=0.12Hz, aromatic), 5.39 (2H, s, CH=CH, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): *δ*= 56 (CH_3), 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 129.4 (C14), 112 (C18, CH), 136 (C19, CH), 127.2 (C20), 127.2 (C21), 114 (C22), 161.2 (C23), 114 (C24); LCMS (m/z): 387.84 $(M)^+$; Anal. Calcd for $C_{23}H_{17}ClN_2O_2$: C, 70.98; H, 4.37; N, 7.20; O, 8.22; Cl, 9.12; Found: C, 70.96; H, 4.34; N, 7.23; O, 8.20; Cl, 9.10.

3-(4-chlorophenyl)-2-[(1E)-3,4,5,6-tetrahydroxyhex-1-en-1-yl]quinazolin-4(3H)-one (QIk): This compound was prepared by the method described above. It was obtained as faint brown crystalline powder, yield: 69%; mp 167-170 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3587 (O-H), 3145 (C-H aromatic), 2967 (=C-H ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1280 (C-N, aromatic), 1100 (C-O aliphatic), 889 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): *δ*= 7.68 (4H, d, *J*=0.56Hz, aromatic), 7.39 (4H, d, *J*=0.12Hz, aromatic), 6.60 (4H, m, *J*=0.91Hz, aromatic), 5.39 (2H, s, CH=CH, ethenylic), 3.78 (3H, s, CH_3), 8.8 (1H, s, OH); ^{13}C NMR ($CDCl_3$, 400 MHz,): *δ*= 165 (C2), 127 (C3), 147.8 (C4), 164

(C6), 128.6 (C7), 129.4 (C14), 114 (C18, CH), 141 (C19, CH), 73.8 (C20), 78.2 (C23), 64.8 (C27); LCMS (m/z): 401.82 $(M)^+$; Anal. Calcd for $C_{20}H_{19}ClN_2O_5$: C, 59.57; H, 4.71; N, 6.95; O, 19.85; Cl, 8.81; Found: C, 59.56; H, 4.73; N, 6.94; O, 19.82; Cl, 8.79.

Procedure for synthesis of *2-[2-(2-Chloro-phenyl)-vinyl]-3-aryl-3H-quinazolin-4-one (QIl-QIt)*
(Synthesis procedure is similar as described above for compounds *QIa-QIk)*

Physical constants and characterization of 2-[2-(2-Chloro-phenyl)-vinyl]-3-aryl-3H-quinazolin-4-one (QIl-QIt)

2-[(E)-2-(2-chlorophenyl)ethenyl]-3-(3-nitrophenyl)quinazolin-4(3H)-one (QIl): This compound was prepared by the method described above. It was obtained as yellow crystalline powder, yield: 70 %; mp 88-93 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3089.9 (C-H, aromatic), 3082 (C=C, ethenylic), 2900 (=C-H, ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1385 (N=O), 1275 (C-N, aromatic), 875 (C-Cl); ^1H NMR ($CDCl_3$, 400 MHz,): δ= 7.85 (4H, d, *J*=0.91, aromatic), 7.4 (4H, m, *J*=0.65Hz, aromatic), 6.90 (4H, d, *J*=0.34Hz, aromatic), 5.5 (2H, s, C<u>H</u>=C<u>H</u>, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 127.1 (C8), 133.2 (C9), 122.1(C10), 139.1(C11), 115.5 (C12), 148.6 (C13), 119.2 (C14), 129.6 (C15), 126.5 (C16), 112 (C18), 136 (C19), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 126.5 (C24), 127.6 (C81.17); LCMS (m/z): 402.83 $(M)^+$; Anal. Calcd for $C_{22}H_{14}ClN_3O_3$: C, 65.37; H, 3.46; N, 7.16; O, 12.28; Cl, 9.08; Found: C, 65.32; H, 3.44; N, 7.10; O, 12.29; Cl, 9.06.

3-(4-bromophenyl)-2-[(E)-2-(2-chlorophenyl)ethenyl]quinazolin-4(3H)-one (QIm): This compound was prepared by the method described above. It was obtained as grey white crystalline powder, yield: 86%; mp 116-119 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3112.3 (C-H aromatic), 2967 (=C-H ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1280 (C-N, aromatic), 691.17 (C-Br), 875 (C-Cl); ^1H NMR ($CDCl_3$, 400 MHz,): δ= 7.49 (4H, d, *J*=0.43Hz, aromatic), 7.81 (4H, d, *J*=0.92, aromatic), 6.69 (4H, d, *J*=0.2Hz, aromatic), 5.54 (2H, s, C<u>H</u>=C<u>H</u>, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 127.1 (C8), 133.2 (C9), 122.1(C10), 137.2 (C11), 122.6 (C12), 132 (C13), 118.7 (C14), 132 (C15), 122.6 (C16), 112 (C18), 136 (C19), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 126.5 (C24), 127.6 (C81.17); LCMS (m/z): 436.71 $(M)^+$; Anal. Calcd for $C_{22}H_{14}BrClN_2O$: C, 60.31; H, 3.19; N, 6.39; O, 3.65; Cl, 16.1; Found: C, 60.32; H, 3.22; N, 6.32; O, 3.63; Cl, 16.11.

2-[(E)-2-(2-chlorophenyl)ethenyl]-3-(3-methylphenyl)quinazolin-4(3H)-one (QIn): This compound was prepared by the method described above. It was obtained as off white crystalline powder, yield: 80%; mp 200-203 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3098.8 (C-H aromatic), 2950 (C-H, CH_3), 2973 (=C-H, ethenylic), 3123 (C=C, ethenylic), 1775 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 879 (C-Cl); ^1H NMR ($CDCl_3$, 400 MHz,): δ= 7.67 (4H, d, *J*=0.56Hz, aromatic), 7.39 (4H, d, *J*=0.87Hz, aromatic), 6.60 (4H, d, *J*=0.87Hz, aromatic), 5.39 (2H, s, C<u>H</u>=C<u>H</u>, ethenylic), 3.26 (3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 20.9 (CH3), 165 (C20, 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 127.1 (C8), 133.2 (C9), 122.1(C10), 138.1(C11), 117.4 (C12), 128.6 (C13), 124.8 (C14), 137.9 (C15), 121.1 (C16), 112 (C18), 136 (C19), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 126.5 (C24); LCMS (m/z): 371.71 $(M)^+$; Anal. Calcd for $C_{23}H_{17}ClN_2O$: C, 74.02; H, 4.55, N, 7.50; O, 4.29; Cl, 9.52; Found: C, 74.04; H, 4.51; N, 7.49; O, 4.30; Cl, 9.45.

2-[(E)-2-(2-chlorophenyl)ethenyl]-3-(4-methoxyphenyl)quinazolin-4(3H)-one (QIo): This compound was prepared by the method described above. It was obtained as orange crystalline powder, yield: 79%; mp 169-171 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3141 (C-H aromatic), 3100 (C-H, CH_3), 2973 (=C-H ethenylic), 1676 (C=C, ethenylic), 1781.17 (C=O), 1650 (C=N, aromatic), 1500 (C=C, aromatic), 1189 (C-O, aliphatic –OCH3), 879 (C-Cl); ^1H NMR ($CDCl_3$, 400 MHz,): δ= 7.67 (4H, m, *J*=1.02Hz, aromatic), 7.39 (4H, t, *J*=0.8Hz, aromatic), 6.60 (t, *J*=0.23Hz, 4H, -CH aromatic), 5.39 (2H, s, CH=CH, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 56 (<u>C</u>H_3), 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 127.1 (C8), 133.2 (C9), 122.1(C10), 130.5(C11), 121.4 (C12), 114.3 (C13), 157.6 (C14), 114.3 (C15), 121.4 (C16), 112 (C18), 136 (C19), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 126.5 (C24); LCMS (m/z): 371.71 $(M)^+$; Anal. Calcd for $C_{23}H_{17}ClN_2O$: C, 74.02; H, 4.55; N, 7.50; O, 4.29; Cl, 9.52; Found: C, 74.03; H, 4.51; N, 7.49; O, 4.27; Cl, 9.51.

2-[(E)-2-(2-chlorophenyl)ethenyl]-3-(2-methoxyphenyl)quinazolin-4(3H)-one (QIp): This compound was prepared by the method described above. It was obtained as white crystalline powder, yield: 60%; m.p.: 189-192 ^oC; IR (KBr) υ_{max}/cm^{-1}: 3249 (C-H, aromatic), 3100 (C-H, CH_3), 2975 (=C-H, ethenylic), 1675 (C=C, ethenylic), 1729 (C=O), 1653 (C=N, aromatic), 1510 (C=C, aromatic), 1200 (C-O, aliphatic –OCH_3), 880 (C-Cl); ^1H NMR ($CDCl_3$, 400 MHz,): δ= 7.45 (4H, t, *J*=0.56Hz, aromatic), 7.2 (4H, d, *J*=0.44Hz, aromatic), 6.75 (4H, t, *J*=0.23Hz, aromatic), 5.4 (2H, s, C<u>H</u>=C<u>H</u>, ethenylic), 3.2 (3H, C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 56 (CH_3), 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 127.1 (C8), 133.2 (C9), 122.1 (C10), 123.0 (C11), 153.9 (C12), 114.3 (C13), 181.17 (C14), 121 (C15), 121.4 (C16), 112 (C18), 136 (C19), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 126.5 (C24), 127.6 (C81.17); LCMS (m/z): 387.84 $(M)^+$; Anal. Calcd for $C_{23}H_{17}ClN_2O_2$: C, 70.98; H, 4.37; N, 7.20; O, 8.22; Cl, 9.12; Found: C, 70.92; H, 4.35; N, 7.23; O, 8.24; Cl, 9.11.

2-[(E)-2-(2-chlorophenyl)ethenyl]-3-(4-nitrophenyl)quinazolin-4(3H)-one (QIq): This compound was prepared by the method described above. It was obtained as yellow crystalline powder, yield: 87 %; mp 111-114 ^{o}C; IR (KBr) υ_{max}/cm^{-1}: 3096.6 (C-H, aromatic), 2901 (=C-H, ethenylic), 1781.17 (C=O), 1681 (C=C, ethenylic), 1660 (C=N, aromatic), 1501 (C=C, aromatic), 1390 (N=O), 1280 (C-N, aromatic), 875 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): δ= 7.29 (4H, d, *J*=0.87Hz, aromatic), 7.45 (4H, t, *J*=1.2Hz, aromatic), 6.61 (4H, d, *J*=0.23Hz, aromatic), 5.34 (2H, s, C<u>H</u>=C<u>H</u>, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 127.1 (C8), 133.2 (C9), 122.1(C10), 144.3 (C11), 121.3 (C12), 123.8 (C13), 144 (C14), 123.8 (C15), 121.3 (C16), 112 (C18), 136 (C19), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 126.5 (C24); LCMS (m/z): 402.81 $(M)^{+}$; Anal. Calcd for $C_{22}H_{14}ClN_3O_3$: C, 65.37; H, 3.46; N, 7.16; O, 12.28; Cl, 9.08; Found: C, 65.33; H, 3.42; N, 7.11; O, 12.24; Cl, 9.05.

2-[(E)-2-(2-chlorophenyl)ethenyl]-3-(2-nitrophenyl)quinazolin-4(3H)-one (QIr): This compound was prepared by the method described above. It was obtained as yellow crystalline powder, yield: 87 %; mp 111-114 ^{o}C; IR (KBr) υ_{max}/cm^{-1}: 3110 (C-H, aromatic), 3083 (C=C, ethenylic), 2910 (=C-H, ethenylic), 1781.17 (C=O), 1660 (C=N, aromatic), 1501 (C=C, aromatic), 1390 (N=O), 1280 (C-N, aromatic), 875 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): δ= 7.29 (4H, d, *J*=0.35Hz, aromatic), 7.45 (4H, d, *J*=0.23Hz, aromatic), 6.61 (4H, t, *J*=0.45Hz, CH, aromatic), 5.34 (2H, s, CH=CH, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 127.1 (C8), 133.2 (C9), 122.1(C10), 133.3 (C11), 140.3 (C12), 123.8 (C13), 181.17 (C14), 134.8 (C15), 121.3 (C16), 112 (C18), 136 (C19), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 126.5 (C24); LCMS (m/z): 402.81 $(M)^{+}$; Anal. Calcd for $C_{22}H_{14}ClN_3O_3$: C, 65.37; H, 3.46; N, 7.16; O, 12.28; Cl, 9.08; Found: C, 65.33; H, 3.42; N, 7.11; O, 12.24; Cl, 9.05.

3-(3-chlorophenyl)-2-[(E)-2-(2-chlorophenyl)ethenyl]quinazolin-4(3H)-one (QIs): This compound was prepared by the method described above. It was obtained as grey crystalline powder, yield: 59%; m.p.: 156-158 ^{0}C; IR (KBr) υ_{max}/cm^{-1}: 3156.4 (C-H, aromatic), 2966 (=C-H, ethenylic), 1772 (C=O), 1651 (C=N, aromatic), 1503 (C=C, aromatic), 1281 (C-N, aromatic), 882 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.29 (t, *J*=0.44, 4H-aromatic), 7.81.17 (t, *J*=0.65, 4H-aromatic), 6.65 (t, *J*=0.34, 4H-aromatic), 5.54 (s, 2H, C<u>H</u>=C<u>H</u>, ethenylic); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 165 (C20, 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 127.1 (C8), 133.2 (C9), 122.1(C10), 139.6 (C11), 120.8 (C12), 134 (C13), 124.5 (C14), 130.1 (C15), 118.5 (C16), 112 (C18), 136 (C19), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 126.5 (C24), 127.6 (C81.17); LCMS (m/z): 392.26 $(M)^{+}$; Anal. calcd. for $C_{22}H_{14}Cl_2N_2O$: C, 67.13; H, 3.55; N, 7.11; O, 4.06; Cl, 18.05; Found: C, 67.11; H, 3.53; N, 7.14; O, 4.05; Cl, 18.07.

3-(2-Chloro-phenyl)-2-[2-(2-chloro-phenyl)-vinyl]-3H-quinazolin-4-one (QIt): This compound was prepared by the method described above. It was obtained as grey crystalline powder, yield: 61%; mp 181-184 ^{o}C; IR (KBr) υ_{max}/cm^{-1}: 3176.3 (C-H, aromatic), 2956 (=C-H, ethenylic), 1752 (C=O), 1654 (C=N, aromatic), 1540 (C=C, aromatic), 1272 (C-N, aromatic), 872 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz,): δ= 7.26 (4H, t, *J*=0.78Hz, aromatic), 7.46 (4H, t, *J*=0.9Hz, aromatic), 6.36 (4H, t, *J*=0.89Hz, aromatic), 5.54 (2H, s, C<u>H</u>=C<u>H</u>, ethenylic); ^{13}C NMR ($CDCl_3$, 400 MHz,): δ= 165 (C2), 127 (C3), 147.8 (C4), 164 (C6), 128.6 (C7), 127.1 (C8), 133.2 (C9), 122.1(C10), 138.6 (C11), 181.17.7 (C12), 129.1 (C13), 181.17.5 (C14), 126.8 (C15), 121.8 (C16), 112 (C18), 136 (C19), 135.3 (C20), 131.5 (C21), 128.8 (C22), 129.1 (C23), 126.5 (C24); LCMS (m/z): 392.26 $(M)^{+}$; Anal. Calcd for $C_{22}H_{14}Cl_2N_2O$: C, 67.13; H, 3.55; N, 7.11; O, 4.06; Cl, 18.05; Found: C, 67.11; H, 3.53; N, 7.14; O, 4.05; Cl, 18.07.

RESULTS AND DISCUSSION

Chemistry

In present research work, synthesis of some newly substituted (3*H*)–quinazolin–4–ones starting from 2–aminobenzoic acid was carried out. A highly employed synthetic method has been used in this synthesis. It involves reaction of 2–amino benzoic acid with acetic anhydride producing 2–methyl–benzoxazin–4–one (step–I) [32, 33]. The product in both reactions is an amide substituted with simple alkyl group which upon ring closure give benzoxazin–4–one. Finally, alkyl substituted quinazolin–4–ones were obtained from the reaction of benzoxazin–4–one with different amines. In obtaining first series of title compounds (QIa–QIk) there is replacement of ring oxygen (step–II) of benzoxazin–4–one with nitrogen atom of 4–chloro aniline. Whereas benzoxazin–4–one containing methyl group at position second was extended further as vinyl (–CH=CH–) linkage (Step–III) joining various substituted aldehydes. It has produced substituted 2–styryl–3-aryl–(3*H*)–quinazolin–4–ones in good quantity (QIa–QIk, Scheme 1).

Similar approach was utilized to obtain second series of title compounds (QIl–QIt) in which there is replacement of benzoxazin–4–one ring oxygen (Step–II) by the nitrogen atom of various substituted amines. Methyl group present at position second of benzoxazin-4-one was linked as vinyl group (–CH=CH–) joining 2–chloro benzaldehyde. It has produced substituted 2–[2–(2–chloro–phenyl)–vinyl]–3–aryl–(3*H*)–quinazolin–4–one in considerable amount (QIl–QIt, Scheme 2). All the crude products were shifted for purification by recrystallization in suitable solvents. The progress of every reaction and formation of product was satisfied with TLC run spotted with raw material and corresponding product.

Synthesis of title compounds (QIa–QIk) and (QIl–QIt) is outlined in Scheme 1 and 2. Typically, experimental procedure (Step–I) is common for both the series of compounds starting with 2–aminobenzoic acid refluxed in anhydrous condition with acetic anhydride. Excess of acetic anhydride was distilled off under reduced pressure and obtained solid was washed twice (2x30mL) with cold water. Crude solid mass was dried in oven and subjected for recrystallization in suitable solvent to obtain 2–methyl substituted benzoxazin–4–one [34]. The formation of benzoxazin–4–one explained by well established mechanism as one mole of the acetic anhydride causes acylation of amine group of 2–aminobenzoic acid and second mole react with the carboxylic group of anthranilic acid leading to formation of a mixed anhydride. This was followed with loss of molecule of acid to give benzoxazinone derivative [35-37].

In relation with (Step–II) of Scheme 1, equimolar mixture of 2–methyl–benzoxazin–4–one and 4–chloro–aniline was reacted under reflux for 6 hours. Free nitrogen atom of aniline can replace the ring oxygen of benzoxazin–4–one and produces 2–methyl–3–aryl–(3*H*)–quinazolin–4–one (QIa–QIk) with release of one molecule of water. In Step–III of Scheme–I, there is reaction of Step–II product 2–methyl–3–aryl–(3*H*)–quinazolin–4–one with various substituted aldehydes under reflux in presence of catalytic amount of glacial acetic acid. This has afforded 2–styryl–3–aryl–(3*H*)–quinazolin–4–one derivatives. Here is extension of 2–methyl group of benzoxazin-4-one in form of ethenyl or vinyl linkage (–CH=CH–) joining to aryl moiety of various aldehydes substituted with significant functional groups. Presence of electron withdrawing and electron donating groups in the amines and aldehydes have possible influence to alter antimicrobial response of compounds under evaluation. Compounds of Scheme II were prepared by reaction of 2–methyl–benzoxazin–4–one with various substituted amines under reflux. It has seemed to replace ring oxygen of benzoxazin–4–one with nitrogen atom of amines producing 3–N–aryl substituted 2–methyl–3–aryl–(3*H*)–quinazolin–4–ones (Step–II). Benzoxazinone with 2–methyl group was linked as vinyl linkage to phenyl moiety of 2–chloro benzaldehyde. The reason behind choice of halogen substituted aldehyde is possible contribution of halogen moiety toward antimicrobial activity. It has afforded 2–(2–chloro–phenyl)vinyl–3–aryl–(3*H*)–quinazolin–4–ones (Step–III, QIl–QIt).

Antimicrobial Screening

The results of MIC (µM x 10^{-3}) finding for all the synthesized compounds are summarized in Table 1. It has shown differential sensitivity of evaluated compounds against microbial strains. Evaluated compounds found to exhibit potent to moderate type of antimicrobial activity against different pathogenic strains of micro–organisms. Collectively excellent antimicrobial activity was observed with MIC range of 9.07 to 10.54 µM x 10^{-3} by compounds as QId, QIf, QIg, QIi, QIj, QIk, QIl, QIm, QIq, QIr, QIs. Moderate antimicrobial activity was observed with MIC range of 16.09 to 22.36 µM x 10^{-3} by the compounds as QIa, QIc, QIe, QIh, QIo, QIn, QIp, QIt. Whereas compound QIb has exhibited least antimicrobial activity (MIC=56.30 µM x 10^{-3}) against evaluated microbial strains. Especially a compound QIi has shown remarkable antimicrobial activity with observed MIC 9.07 µM x 10^{-3} as compared to Ciprofloxacin (18.08 µM x 10^{-3}) and Griseofulvin (19.25 µM x 10^{-3}).

Table-1: Antimicrobial activity of 3–(4–chloro–phenyl)–2–styryl–(3*H*)–quinazolin–4–one (QIa–QIk) (Scheme 1) and 2–[2–(2–chloro–phenyl)–vinyl]–3–aryl–(3*H*)–quinazolin–4–one (QIl–QIt) (Scheme 2)

Comp	Minimum inhibitory concentration (µM x 10^{-3})[a]						Comp	Minimum inhibitory concentration(µM x 10^{-3})[a]					
	Gram +ve bacteria		Gram –ve bacteria		Fungi			Gram +ve bacteria		Gram –ve bacteria		Fungi	
	S.a	*B.s*	*K.p*	*P.a*	*C.a*	*A.n*		*S.a*	*B.s*	*K.p*	*P.a*	*C.a*	*A.n*
QIa	20.48	20.48	20.48	20.48	20.48	20.48	QIl	10.50	10.50	10.50	10.50	10.50	10.50
QIb	56.30	56.30	56.30	56.30	56.30	56.30	QIm	11.38	11.38	11.38	11.38	20.29	20.29
QIc	20.95	20.95	20.95	20.95	20.95	20.95	QIn	16.09	16.09	16.09	16.09	16.09	16.09
QId	10.54	10.54	10.54	10.54	10.54	10.54	QIo	19.24	19.24	19.24	19.24	19.24	19.24
QIe	20.24	20.24	20.24	20.24	20.24	20.24	QIp	20.07	20.07	20.07	20.07	20.07	20.07
QIf	10.23	10.23	10.23	10.23	19.50	19.50	QIq	10.49	10.49	10.49	10.49	10.49	10.49
QIg	10.48	10.48	10.48	10.48	10.48	10.48	QIr	10.49	10.49	10.49	10.49	10.49	10.49
QIh	22.36	22.36	22.36	22.36	22.36	22.36	QIs	10.22	10.22	10.22	10.22	19.41	19.41
QIi	9.07	9.07	9.07	9.07	9.07	9.07	QIt	21.40	21.40	21.40	21.40	21.40	21.40
QIj	10.11	10.11	10.11	10.11	10.11	10.11	---	----	----	----	----	----	----
QIk	10.48	10.48	10.48	10.48	10.48	10.48	----	----	----	----	----	----	----
CPF	18.08	18.08	18.08	18.08	---	---	CPF	18.08	18.08	18.08	18.08	---	---
GS	---	---	---	---	19.25	19.25	GS	---	---	---	---	19.25	19.25

[a]The lowest concentration of test compound needed for prevention of visible growth of microorganism.

[b]Micro–organisms– *S.a*; *Staphylococcus aerues, B.s*; *Bacillus subtilis, K.p*; *Kleibsella pneumoniae, P.a*; *Pseudomonas aeruginosa, C.a*; *Candida albicans, A.n*; *Aspergillus niger*, CPF; Ciprofloxacin (50µg/ml), GS; Griseofulvin (100µg/ml).

In a nutshell of these all above MIC findings, maximum number of evaluated compounds has shown considerable positive activity in comparison with positive control standards Ciprofloxacin and Griseofulvin for bacteria and fungi respectively. The overall results of described biological study signify importance of 2,3–disubstituted–(3*H*)–quinazolin–4–ones as novel scaffolds bearing functional groups sharing antimicrobial activity. In addition, the compounds especially QIi (MIC, 9.07 µM x 10^{-3}) and QIj (MIC, 10.11 µM x 10^{-3}) with least MIC amongst all evaluated compounds considered as lead molecules and selected for further design and development of novel and effective antimicrobial agents. The search for novel, effective, and safe antimicrobial agents is a major thrust area in the mainstream of antimicrobial research. The results of biological activity also hold importance of the eve of multi drug resistance (MDR) crisis of pathogenic micro–organism.

SAR for Antimicrobial Activity

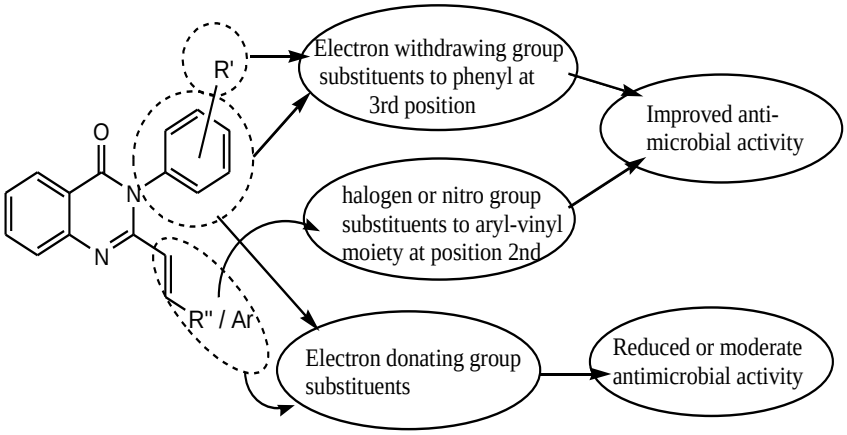


Fig-2: Structure activity relationship for antimicrobial activity

From structure activity relation (SAR) point of view, it is known to affect biological activity differently by the substituents at varying positions. Electro–negative substituents attached to the phenyl group at position two and styryl group at position three of quinazoline nucleus may responsible to enhance antimicrobial potency. However, electro–positive substituents at these positions are detrimental for activity (Figure 2). For instance, substitution of (3*H*)–quinazoline–4–one nucleus with amine nitrogen atom at position three and joining of vinyl linked aldehydes at position two is one factor for variation in MIC of title compounds. Another factor for contribution of excellent antimicrobial activity is substitution of quinazoline–4–one nucleus with aldehydes and amines bearing electron withdrawing groups like –Cl, –Br, $–NO_2$, –OH, $–NH_2$ at their respective positions. In addition, presence of vinyl linked aryl substituents may responsible to promote antimicrobial activity of title compounds. Antimicrobial response was reduced because of substitution of quinazoline–4–one nucleus with electro–positive groups like $–CH_3$, OCH_3.

CONCLUSIONS

All the synthesized compounds were screened for *in–vitro* antimicrobial activity against different pathogenic microbial strains of bacteria and fungi. It was observed that compounds like QId, QIf, QIg, QIi, QIj, QIk, QIl, QIm, QIq, QIr, QIs found to exhibit excellent antimicrobial activity as compared to positive control antibiotics Ciprofloxacin and Griseofulvin (Table 1). Structure activity correlation of quinazolin–4–one compounds revealed that scaffold molecules having substituents with good electron withdrawing ability at position two and three has shown significant antimicrobial activity. In addition, molecules bearing electron donating groups were observed with comparative less activity.

Close and equipotent activity was exhibited by the compounds as QIa, QIc, QIe, QIp (MIC, 20.07–20.95 µg/ml). However, compounds as QId, QIf, QIg, QIj, QIk, QIl, QIq, QIr, QIs (MIC, 10.11–10.54 µg/ml) has shown equipotent activity amongst each other but comparative more potent than the standard drugs Ciprofloxacin and Griseofulvin (MIC, 18.08 and 19.25 µg/ml respectively). Surprisingly, one of compound QIi has exhibited highest activity (MIC, 9.07) than the standards used for activity. The result outcome of antimicrobial activity has formed a foundation base for further investigations toward antimicrobial activity in our laboratories. The lead motifs in the biological activity would be further explored as active antibiotics against treatment of deadly infections.

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