Synthesis and Antimicrobial Study of Pyrimidinone Substituted 4(3H)-Quinazolinone Derivatives

Natvar A Sojitra¹, Rajesh K Patel¹, Ritu B Dixit² and Bharat C Dixit*²

¹Chemistry Department, V. P. & R. P. T. P. Science College, Affiliated to Sardar Patel University, Gujarat, India
²Ashok & Rita Patel Institute of Integrated Studies and Research in Biotechnology and Allied Sciences, Gujarat, India

Abstract

A series of structurally diverse and newly designed pyrimidinone substituted 4(3H)-Quinazolinone derivatives 6a–6j were synthesized in a simple and facile manner under both conventional and microwave heating conditions. Entitled compounds 6a–6j were synthesized using N-acylanthranilic acid derivatives 1a–1j and hydrazinylbenzensulfonamide 2 as a key starting materials, which under goes hierarchy of the reactions via different intermediate stages; quinazolinone derivatives 3a–3j and hydrazonoquinazolinone derivatives 5a–5j. All the synthesized compounds are in good amount of yield. The structure of entitle compounds have been evaluated on the basis of various spectroscopic techniques and analytical methods as well as, all the synthesized compounds were subjected to in vitro antibacterial activities. Some of the compounds displayed moderate to good in vitro antimicrobial activity by broth micro dilution method against pathogenic bacteria (S. aureus, B. subtilis, B. megaterium, E. coli, P. vulgaris, P. aeruginosa) species.

Keywords: Quinazolinone; Pyrimidinone; Antimicrobial activity; Microwave heating

Introduction

Heterocyclic scaffold has been under discovery due to their considerable therapeutic and medicinal importance. One of such heterocycles 2-pyrimidinone, while not naturally occurring, are receiving increased attention because of their wide-ranging biological activity like, an efficient inhibitor of DNA synthesis in E. coli, inhibitors of the enzyme cytidinedeaminase, and as magistrate inhibitors. Such pyrimidinone derivatives and the pharmaceutical acceptable salts thereof having remarkable antagonistic action against angiotensin II receptor, thereby, being useful in treating cardiovascular disease caused by binding angiotensin II to its receptor. Antitumor activity of pyrimidinones derivatives have shown statistically significant synergism with cyclophosphamide (CY) against antitumor / P388 leukemia etc. [1-7]. They have also shown anticanic [8,9], potent TF-FVIIa inhibitors [10] and inhibition of C5-cytosine DNA methyltransferases [11] activities.

In addition to that, quinazolinone and its derivatives comprise an important class of heterocyclic molecules due to their broad spectrum application in medicinal and pharmaceutical chemistry such as analogic [12], antimicrobial and antitubercular [13,14], antitumor [15], anticanic [16], anti-inflammatory [17], anticonvulsant [18], antimalarial and antihistamine [19].

Moreover, there are many reports regarding synthesis of pyrimidinone derivatives [20,21] but no reports are available in which pyrimidinone is substituted at 3rd position of quinazolinone through hydrazono benzensulfonamide linkage. Looking to the aforementioned literature and our ongoing research in the field of quinazolinone derivatization [22,23], we reports here synthesis of various pyrimidinone substituted quinazolinone derivatives linked through hydrazono benzensulfonamide, of that some of the compounds showed good to moderate antimicrobial activity.

Experimental Section

Material and measurements

All the chemicals were purchased from Spectro chem. Ltd. (Mumbai, India) and were used without further purification. Solvents employed were distilled, purified and dried by standard procedures prior to use [24]. Melting points of the synthesized compounds were determined in open capillary tube method and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC on aluminum plates coated with silica gel 60 F254, 0.25 mm thickness purchased from E. Merck Ltd., Mumbai-India). The mobile phase was chloroform and methanol (9:1), and detection of the components was done under UV light or explore in Iodine chamber. Infrared (IR) spectra were recorded as potassium bromide pellets using a Shimadzu 8501 Fourier transform infrared (FTIR) Spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Bruker AVANCE II 400-MHz NMR spectrometer (Bruker Corporation, Billerica, MA, USA), with chemical shift in ppm downfield from TMS as an internal reference and DMSO-d⁶ solvent. Carbon, hydrogen and nitrogen elemental analysis were estimated by PerkinElmer 2400-II CHN elemental analyzer, USA. The solvent. Carbon, hydrogen and nitrogen elemental analysis were estimated by PerkinElmer 2400-II CHN elemental analyzer, USA. The solvent. Carbon, hydrogen and nitrogen elemental analysis were estimated by PerkinElmer 2400-II CHN elemental analyzer, USA. The solvent.

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General procedure for the synthesis of 4-amino-N-(2-methyl/aryl-4-oxoquinazolin-3(4H)-yl)benzenesulfonamide derivatives (3a–3j)

Compounds 3a–3j were prepared according to the reported method [22,23]. The possible synthetic route is given in Scheme 1.

-3-yI)benzenesulfonamide (5b) m.p. 145–147°C, ESI MS (m/z): 520.1, 522.3 [M]+.

4-[N'-(1-Acetyl-2-oxo-propylidene)hydrazino]-N-(6,8-dibromo-2-methyl-4-oxo-4H-quinazolin-3-yl)benzenesulfonamide (5c) m.p. 172–174°C, ESI MS (m/z): 597.2, 599.1, 601.3 [M]+.

4-Amino-N-(2-methyl-4-oxoquinazolin-3(4H)-yl)benzenesulfonamide (3a) mp 217-218°C. ESI MS (m/z): 330.8 [M+H]+.

4-Amino-N-(6-bromo-2-methyl-4-oxoquinazolin-3(4H)-yl)benzenesulfonamide (3b) mp 187-189°C. ESI MS (m/z): 409.2, 411.3 [M]+.

4-Amino-N-(6,8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-yl)benzenesulfonamide (3c) mp 212-214°C. ESI MS (m/z): 488.4, 490.4, 492.4 [M]+.

4-Amino-N-(6-nitro-4-oxo-2-phenylquinazolin-3(4H)-yl)benzenesulfonamide (3d) mp 166-168°C. ESI MS (m/z): 393.4 [M+H]+.

4-Amino-N-(6-bromo-4-oxo-2-phenylquinazolin-3(4H)-yl)benzenesulfonamide (3e) mp 175-179°C. ESI MS (m/z): 426.9, 428.9 [M]+.

4-Amino-N-(6,8-dibromo-4-oxo-2-phenylquinazolin-3(4H)-yl)benzenesulfonamide (3f) mp 175-179°C. ESI MS (m/z): 426.9, 428.9 [M]+.

4-Amino-N-(6-bromo-4-oxo-2-phenylquinazolin-3(4H)-yl)benzenesulfonamide (3g) mp 219-221°C. ESI MS (m/z): 548.3, 550.3, 552.3 [M]+.

4-Amino-N-[2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl]benzenesulfonamide (3h) mp 173-179°C. ESI MS (m/z): 426.9, 428.9 [M]+.

4-Amino-N-[6-bromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl]benzenesulfonamide (3i) mp 173-179°C. ESI MS (m/z): 426.9, 428.9 [M]+.

Scheme 1: Proposed synthetic route for the preparation of pyrimidinone substituted 4(3H)-quinazolinone derivatives compound (6a-6j).
3(4H)-yl]benzene-sulfonamide (3i) mp 186–187°C. ESI MS (m/z): 503.8, 505.8, 507.8 [M]+.

4- Amino-N- [6,8-dibromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl]benzene-sulfonamide (3j) mp 183–184°C. ESI MS (m/z): 582.5, 584.5, 586.5, 588.5 [M]+.

**General procedure for the diazotization of 4-amino-N-(2-methyl/aryl-4-oxoquinazolin-3(4H)-yl)benzenesulfonamide derivatives (4a-4j)**

The diazotized derivatives 4a-4j were prepared by following the reported method [23,25]. Schematic diagram to produce diazotized compounds 4a-4j are shown in Scheme 1. The reaction mass was cooled to 0-5°C for 1 hour and was directly used for the next step.

**General procedure for the synthesis of hydrazono derivatives of compounds 4a-4j using acetyl acetone as an active methylene compound (5a-5j)**

Hydrazonoquinazolinone compounds 5a-5j were prepared by following the reported method [23,26]. The synthetic route is shown in Scheme 1.


- **4-[N’-(1-Acetyl-2-oxo-propylidene)hydrazino]-N-[6-bromo-2-methyl-4-oxo-4H-quinazolin-3(4H)-yl]benzenesulfonamide (5b)** mp 183–184°C, ESI MS (m/z): 582.5, 584.5 [M]+.


- **4-[N’-(1-Acetyl-2-oxo-propylidene)hydrazino]-N-[5-(2-methyl-4-oxo-2-phenyl-4H-quinazolin-3(4H)-yl)benzenesulfonamide (5e)** mp 203–204°C, ESI MS (m/z): 616.2, 617.9, 620.0 [M]+.

- **4-[N’-(1-Acetyl-2-oxo-propylidene)hydrazino]-N-[5-(4,6-dimethyl-2-oxopyrimidin-5(2H)-ylidene)hydrazinyl]-(2-methyl-4-oxo-4H-quinazolin-3(4H)-yl)benzenesulfonamide (5f)** mp 178–180°C, ESI MS (m/z): 538.0, 539.8 [M]+.

- **4-[N’-(1-Acetyl-2-oxo-propylidene)hydrazino]-N-[5-(4,6-dimethyl-2-oxopyrimidin-5(2H)-ylidene)hydrazinyl]-(2-methyl-4-oxo-4H-quinazolin-3(4H)-yl)benzenesulfonamide (5g)** mp 203–204°C, ESI MS (m/z): 616.2, 617.9, 620.0 [M]+.


**Synthesis of pyrimidinone substituted quinazolinone derivatives 6a–6j**

It was prepared using the appropriate hydrazono compound 5a–5j (0.01 mol) and urea (0.015 mol) in 40 ml absolute ethanol using anhydrous sodium acetate as a catalyst (0.001 mol). The reaction mixture was heated under reflux for 5 hours. Completion of reaction was checked by TLC using 9:1 CHCl₃:CH₃OH solvent system. After cooling to room temperature the reaction mixture was poured into crushed ice and the mixtures was stirred for 1 hours. Separated product was collected by filtration, washed with water, dried and recrystallized from aqueous ethanol. The colors of the compounds were yellowish to dark orange and are soluble in polar organic solvents like methanol, ethanol and pyridine. The synthetic route is shown in Scheme 1. Yield was in the range of 55-68%.

**General microwave assisted procedure for the synthesis of pyrimidinone substituted quinazolinone derivatives 6a–6j**

Appropriate hydrazono compound 5a–5j (0.01 mol), urea (0.015 mol), anhydrous sodium acetate (0.001 mol) and 2 ml ethanol were taken in a two-neck round bottomed flask fitted with a device condenser. The mixture was then heated under microwave irradiation at 350 W for 3-5 minutes. After cooling to room temperature the reaction mixture was poured into crushed ice and the mixtures was stirred for 30 minutes. Separated product was collected by filtration, washed with water, dried and recrystallized from aqueous ethanol. The colors of the compounds were yellowish to dark orange and are soluble in polar organic solvents like methanol, ethanol and pyridine. Yield was in the range of 71-83%.

**4-[N’-(4,6-Dimethyl-2-oxo-2H-pyrimidin-5-ylidene)hydrazino]-N-[2-methyl-4-oxo-4H-quinazolin-3(4H)-yl]benzenesulfonamide (6a)**

Off white solid (Yield 76%).Mp: 145-147°C. IR (KBr): 3108 (C=O), 1697 (C=N), 1336, 1154 (S=O) cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆) δ 10.34 (s, 2H, -NH₂), 7.97 (dd, J = 8.01, 1.52 Hz, 1H, Ar-H), 7.11 (dd, J = 11.75, 1.11 Hz, 1H, Ar-H), 6.87 (d, J = 7.88, 1.11 Hz, 1H, Ar-H). 13C NMR (100 MHz, DMSO-d₆) δ 123.83, 122.29, 115.11, 24.7, 23.08. MS m/z 588.5 [M]+.
N-(2-(4,6-dimethyl-2-oxopyrimidin-5(2H)-ylidene) hydrazinyl)-N-(4-methyl-6-nitro-4-oxoquinazolin-3(4H)-yl) benzenesulfonamide (6d)

Bright yellow (Yield 75%). Mp: 194-195°C. IR (KBr): 3110 (C-H Ar), 1694 (C=O), 1601 (C=N), 1337, 1153 (S=O) cm-1. 1H NMR (400 MHz, DMSO-d6) δ 10.79 (s, 1H, SO_NH), 7.83 (dd, J = 17.1, 15.6 Hz, 1H, Ar-H), 7.49 (dd, J = 7.88, 1.41 Hz, 1H, Ar-H), 7.37 (td, J = 7.17, 1.40 Hz, 1H, Ar-H), 7.24 (dd, J = 8.64, 2.63 Hz, 1H, Ar-H), 6.87 (dd, J = 10.68, 2.14 Hz, 2H, Ar-H), 2.48 (s, 6H, CH3). 13C NMR (400 MHz, DMSO-d6) δ 171.32, 159.11, 159.11, 158.93, 154.63, 154.88, 152.21, 144.61, 141.21, 136.21, 130.21, 127.43, 124.71, 131.11, 24.10, 22.83. MS m/z: 511 (M+). Anal. Calcd for C26H18N8O6S: C-49.41, H-3.31, N-22.09, S-6.47. Found: C-49.22, H-3.31, N-22.09, S-6.47.

4-[(N-(6-bromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-4-(2-(4,6-dimethyl-2-oxopyrimidin-5(2H)-ylidene) hydrazinyl)]-N-(4-oxo-2-phenyl-4H-quinazolin-3-yl) benzenesulfonamide (6e)

Creamy white solid (Yield 71%). Mp: 215-217°C. IR (KBr): 3110, 1689 (C=O), 1601 (C=N), 1337, 1153 (S=O) cm-1. 1H NMR (400 MHz, DMSO-d6) δ 10.41 (s, 1H, SO2NH), 8.37 (s, 1H, -NH-), 7.71 (dd, J = 7.71, 1.56 Hz, 1H, Ar-H), 7.54 (m, 4H, Ar-H), 7.38 (dd, J = 7.88, 1.41 Hz, 1H, Ar-H), 7.37 (td, J = 7.17, 1.56 Hz, 1H, Ar-H), 7.24 (dd, J = 8.64, 2.63 Hz, 1H, Ar-H), 6.87 (dd, J = 10.68, 2.14 Hz, 2H, Ar-H), 2.21 (s, 6H, CH3). 13C NMR (400 MHz, DMSO-d6) δ 173.32, 164.58, 155.11, 153.65, 152.85, 149.75, 144.65, 137.83, 136.27, 132.17, 130.55, 129.41, 128.61, 126.61, 126.02, 125.24, 122.66, 113.76, 23.19. MS m/z: 563 (M+). Anal. Calcd for C26H17Cl2N7O4S: C-45.35, H-2.96, N-14.18, S-4.77. Found: C-45.35, H-2.96, N-14.18, S-4.77.

N-(6-bromo-2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl)-4-(2-(4,6-dimethyl-2-oxopyrimidin-5(2H)-ylidene)hydrazinyl) benzenesulfonamide (6f)

Pale yellow (Yield 79%). Mp: 188-190°C. IR (KBr): 3104 (C=O), 1698 (C=O), 1606 (C=N), 1337, 1155 (S=O) cm-1. 1H NMR (400 MHz, DMSO-d6) δ 10.47 (s, 1H, SO2NH), 8.31 (s, 1H, -NH-), 7.83 (dd, J = 7.71, 1.56 Hz, 1H, Ar-H), 7.49 (m, 3H, Ar-H), 7.38 (d, J = 7.88, 1.41 Hz, 1H, Ar-H), 7.26 (d, J = 7.88 Hz, 2H, Ar-H), 6.85 (d, J = 7.88 Hz, 1H, Ar-H), 6.72 (dd, J = 8.64, 2.63 Hz, 1H, Ar-H), 6.41 (dd, J = 10.68, 2.14 Hz, 2H, Ar-H), 2.18 (s, 6H, CH3). 13C NMR (400 MHz, DMSO-d6) δ 172.67, 161.32, 159.11, 155.41, 152.35, 147.63, 142.14, 137.51, 136.71, 135.57, 134.17, 132.43, 130.64, 129.86, 123.81, 123.09, 114.12, 113.59, 23.22. MS m/z: 641 (M+). Anal. Calcd for C26H16Br2N7O4S: C-48.73, H-2.99, N-15.30, S-5.00. Found: C-48.86, H-2.73, N-15.42, S-5.17.

In vitro antimicrobial studies

All the synthesized compounds were screened for their in vitro antimicrobial activities against selected pathogenic bacterial strains to determine minimum inhibitory concentrations (MIC) by broth micro dilution method [27] using Sulfanilamide as reference drugs of parent moieties, while Streptomycin was employed as a standard antibacterial drug.

The in vitro antimicrobial activities of all the synthesized compounds were screened for their antibacterial against Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium (Gram Positive) and Escherichia coli, Proteus vulgaris, Pseudomonas aeroginosa (Gram
Negative), using the broth dilution method [27]. All the ATCC culture was collected from institute of microbial technology, Bangalore. 2% Luria broth solution was prepared in distilled water while, pH of the solution was adjusted to 7.4 ± 0.2 at room temperature and sterilized by autoclaving at 15 lb pressure for 25 min. The tested bacterial and fungal strains were prepared in the lura broth and incubated at 37°C and 200 rpm in an orbital incubator for overnight. Sample solutions were prepared in DMSO for various concentration. The standard drug solution of Streptomycin (antibacterial drug) was prepared in DMSO. Serial broth micro dilution was adopted as a reference method. 10 μL solution of test compound was inoculated in 5 ml lura broth for each concentration respectively and additionally one test tubes was kept as control. Each of the test tubes was inoculated with a suspension of standard microorganism to be tested and incubated at 35°C for 24 h. At the end of the incubation period, the tubes were examined for the turbidity. Turbidity in the test tubes indicated that microorganism growth has not inhibited by the antibiotic contained in the medium at the test concentration. The antimicrobial activity tests were performed in triplicate and the deviation for any triplicate results was not more than ± 1% to 5% while average MIC values of the compounds are represented in Table 1.

Results and Discussion

The intention of the present study was to synthesize and investigate the antimicrobial activities of pyrimidinone ring substituted at 4(3H)-quinazolinone derivatives (6a-6j) through hydrazono benzenesulfonylamine linkage in a single molecular frame work. Synthetic rout of the target molecules is outlined in the Scheme 1 under both conventional and microwave heating condition. When cyclization for the formation of pyrimidinone derivatives was performed under microwave irradiation, the reaction was taken place in 3 minutes in an improved yield.

All the synthesized compounds were characterized by their physical, analytical and spectral properties. The IR, ESI-MS and NMR (1H and 13C) spectral data of all the synthesized compounds were in good agreement with the structure assigned. Further, in the MS-EI with positive mode spectra exhibited molecular ion peak ([M+H]+), appeared at different intensities, confirmed the exact mass or molecular weights of the examined compounds 6a-6j, while appearance of a characteristic two isotope peak ([(M+H)+2]) along with molecular ion peak ([(M+H)+]) in an intense ratio almost 3:1 or 1:1 to the molecular ion peak confirmed the presence of halogen (Cl or Br) atoms of high abundance nature. The IR spectrum of 6a-6j showed the presence of C=O group at ~1690 cm⁻¹ due to CONH and COCH₃ groups. Two sharp bands at ~1340 and ~1160 cm⁻¹ were due to asymmetric and symmetric stretching vibrations of SO₂ group respectively. The ¹H NMR of entitled compounds 6a-6j showed singlet around δ ~10.50 ppm due to highly deshielded proton of –NHSO₂ group. One singlet was observed about δ ~8.77 ppm due to secondary amine group. The two more singlet appeared in the aliphatic region at δ 2.43 and 2.18 ppm due to –CH₂(second position of the quinazolinone ring) and –CH₃ (two methyl group of pyrimidinone ring) protons. All the eight aromatic protons resonated in the region of δ 7.79 to 6.44 ppm. The ¹³C NMR of final compounds 6a-6j showed two signals of carbonyls carbon around δ ~170 (CO of pyrimidinone ring), ~160 (CO of quinazolinone ring). The aromatic carbons appeared between δ ~113-158 ppm. The signals due to aliphatic carbons appeared about ~24 and ~22.5 ppm due to presence of two different methyl groups in quinazolinone ring and pyrimidinone ring.

In vitro antimicrobial activities

Inspection of the data of Table 1, on the preliminary in vitro antimicrobial evaluations of the entitled compounds 6a-6j revealed that all the screened compounds have a varied degree of antibacterial activity, evident from their MIC values in μg/mL. Among the screened compounds, most of the compounds have shown more or equal antimicrobial activities compare to the reference drug Sulfanilamide (Sf), while a very few of the screened compounds found to be equipotent to the standard drugs, Streptomycin (Sm). From the results of in vitro antibacterial activity data, it was observed that compounds 6e, 6h, 6i and 6j demonstrated excellent activity against E. coli and P. vulgaris bacterial species. In general, compounds showed more selectivity against Gram negative over Gram positive species amongst all the bacterial strains. All the compounds were screened for their in vitro antimicrobial activities and results revealed that the group 4-chlorophenyl substitution at the 2nd position in quinazolin-4(3H)-one nucleus led to increase their biological activities.

Conclusion

In summary, a series of new pyrimidinone substituted Quinazolin-4(3H)-one derivatives have been synthesized by adapting a simple and facile manner using both conventional and microwave heating condition in a good amount of yields. All the compounds were screened for their in vitro antimicrobial activity and results revealed

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<tr>
<th>Compound</th>
<th>Minimum Inhibition Concentration in µg/mL</th>
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<td>Gram positive bacteria</td>
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<td>Sm</td>
<td>425</td>
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Table 1: Antimicrobial activities of the synthesized compounds 6a-6j.

*Sf= Sulfanilamide, Sm = Streptomycin

that compounds 6e, 6h, 6i and 6j demonstrated excellent activity against *E. coli* and *P. vulgaris* bacterial species.

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References