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Design and synthesis of quinazolinone derivatives as a novel antitubercular agents

Meenu Chaudhary*[1], S. Bhattacharya[2], Yusra Ahmad[3]

*[*1]Division of Pharmaceutical Sciences, S.G.R.R.I.T.S. Patel Nagar, Dehradun, Uttrakhand, India*
[2]Department of Pharmaceutical Chemistry, SIP, Allahabad, Utter Pradesh, India
[3]Department of Pharmacology, Uttrakhand Technical University, Dehradun, Uttrakhand, India

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ABSTRACT

*Quinazolinone is a compound made up of two fused six member simple aromatic rings-benezene and pyrimidine ring and have been reported to posses versatile type of biological activities such as anticancer, anticonvulsant, anti-inflammatory, antihelminthic, antimicrobial activities. A series of novel substituted-[1,2,4]triazolo[1,5c]quinazolinone derivatives (K11-19) were synthesized by mannich reaction using formamide and different secondary amines. Structures of compounds synthesized were confirmed by IR, ^{1}H-NMR and Mass spectroscopic analysis. All synthesized compounds were screened for anti-tubercular activity. The anti-tubercular activity of synthesized compounds was performed against M. tuberculosis H37Rv at concentration 30 µg/ml using Streptomycin and Pyrazinamide 7.5 µg/ml as standard drug. All synthesized compounds have shown anti-tubercular activity as compared to standard drug Streptomycin, Pyrazinamide. Compounds **K 13** and **K 17** have shown very good anti-tubercular activity.*

Key words: [1,2,4]triazolo[1,5c]quinazolinone derivatives; anti-tubercular activity; mannich reaction.

INTRODUCTION

Quinazolinone is the major fused six-member heterocyclic ring system and is one of the most encountered heterocyclic in medicinal chemistry and a building block for around 120 naturally occurring alkaloids.

Quinazolinone constitute an important class of medicinally important small molecules which have been reported to possess anticonvulsant [1-3], antimicrobial [4-8], anti-inflammatory [9-11], antitumor [12], anticancer [13], sedative-hypnotic [14], diuretic [15-16], antiviral [17], antihypertensive [18] and antitubercular [19-20] activities. Several 2, 3-disubstituted Quinazolinone derivatives were synthesized and tested for different biological activities. These reports showed that aryl substitution at 2nd and 3rd position enhances biological activities.

Efforts towards the development and identification of new molecules for anti-tubercular activities with minimal side effects have gained significance in the recent past during which the quinazolinones came into the scenario.
With the revelation of exploring the diverse pharmacological nature of [1,2,4]triazolo[1,5c]quinazolinone derivatives, it was contemplated to synthesize some substituted quinazolinone derivatives by mannich reaction having general structure of figure 1 as potential anti-tubercular agents.

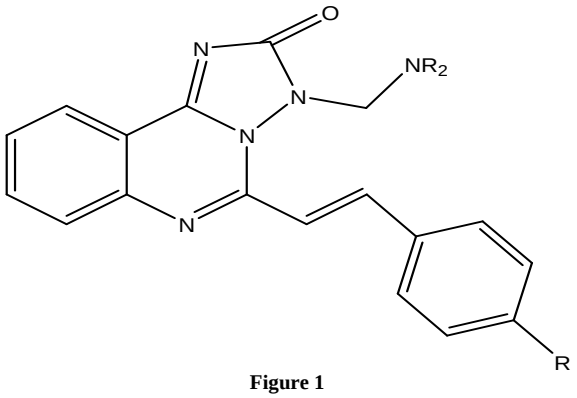


Figure 1

MATERIALS AND METHODS

Chemistry: Substituted-[1,2,4] Triazolo[1,5c]Quinazolinone derivatives were synthesized by five steps. 2-methyl-benzoxazin-4-one was prepared by using acetic anhydride. Treating it with semicarbazide to produce corresponding (2-methyl-4-oxo-quinazolin-3-yl)-urea; which further heated above its melting to get 5-methyl-[1,2,4]triazolo[1,5c]quinazolin-2-one respectively. To the stirred solution of an equivalent amount of 5-methyl-[1,2,4]triazolo[1,5c]quinazolin-2-one and appropriated benzaldehyde in ethanol was added aqueous NaOH solution(10% w/v, 10ml). The title compounds could be obtained by mannich reaction using formamide and different secondary amines.

Melting points were recorded in open capillaries with electric melting point apparatus and were uncorrected. IR spectra (KBr disks) were recorded using Shimadzu 8400S FTIR spectrophotometer. ^{1}H-NMR were recorded in Bruker Avance II(400 MHz) spectrophotometer in $CDCl_3$ solution and chemical shift values were reported in ppm relative to TMS(δ = 0) as internal standard. Mass spectra were recorded on a Shimazu LC-MS (2010A) spectrophotometer. TLC was performed on silica gel coated plates for monitoring the reactions.

Preparation of 2-methyl-benzoxazin-4-one:
A mixture of (6.8 g, 0.1 mole) anthranilic acid and acetic anhydride(11 ml, 0.02 mole) was refluxed for 6 hours and while hot poured into cold water. Allow the reaction mixture to cool at room temperature then washed with methanol, residue was dried at room temperature further recrystallized with methanol and dried.

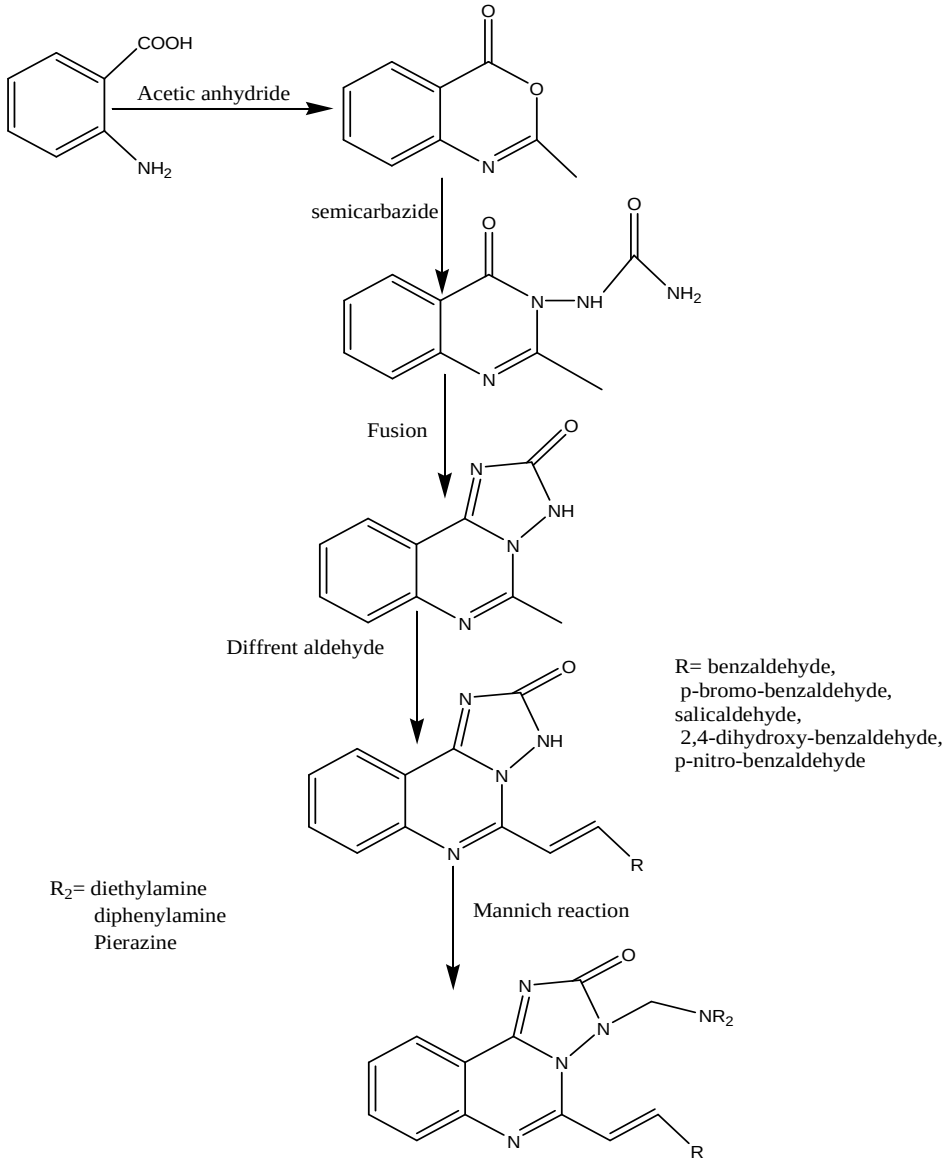
Synthesis of (2-Methyl-4-oxo-4H-quinazolin-3-yl)-urea:
An equi-molar quantity (8.5 g, 0.05 mole) of 2-methyl-benzoxazin-4-one and semicarbazide were dissolved in ethanol separately. Then, mixture was refluxed for 1 hour with glacial acetic acid. After the completion of reaction, contents were cooled to room temperature. Solid mass was collected and recrystallized with methanol.

Synthesis of 5-methyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one:
The product obtained from step 2 was heated above its melting point to get 5-methyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one. Product was recrystallized with methanol.

Synthesis of substitued-[1,2,4]triazolo[1,5c]quinazolin-2-one:
An equimolar mixture (0.01mole) of 5-methyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one and substituted benzaldehyde was fitted with mechanical stirrer, and solution was immersed into cold water bath. To this, 10% of NaOH solution was added slowly until the mixture become just acidic to litmus. The reaction mixture was poured into ice cold water. The solid so obtained was filtered, dried and recrystallized with methanol.

Synthesis of title quinazolinone derivatives (K 11- K 19):
A slurry consisting of 5-substituted-[1,2,4]triazolo[1,5-c]quinazolin-2-one, ethanol (5ml) and 37% formalin (1ml) was made. To this added different secondary amine (0.01mole) drop wise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1 hour with occasional shaking, after which it was warmed on steam bath for 15 minutes. At the end of the period the contents were cooled and recrystallized from chloroform and petroleum ether.

RESULTS AND DISCUSSION

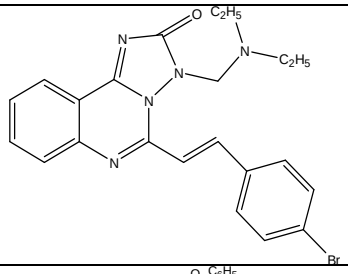
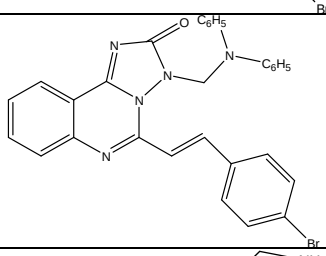
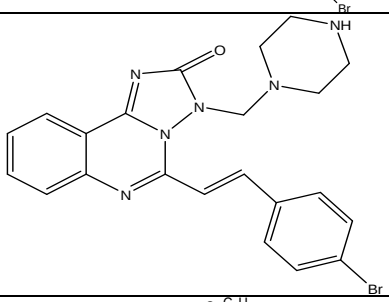
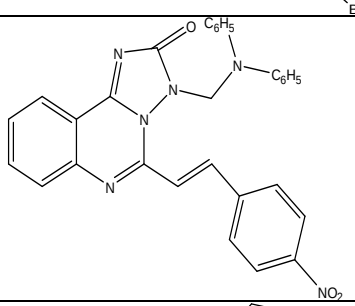
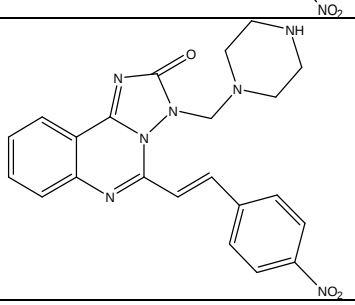


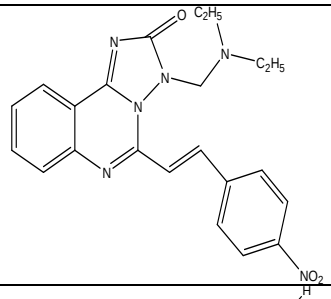
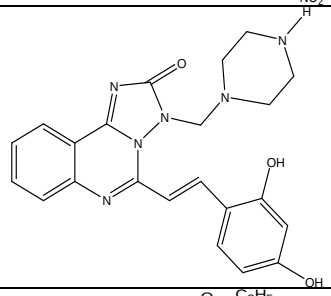
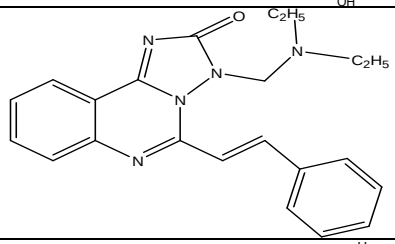
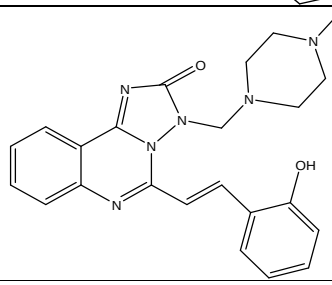
Scheme1

The synthesis of title compound contains five steps. 2-methyl-benzoxazin-4-one was prepared by using acetic anhydride [21]. Treating it with semicarbazide to produce corresponding (2-methyl-4-oxo-quinazolin-3-yl)-urea; which further heated above its melting to get 5-substitued-[1,2,4]triazolo[1,5c]quinazolin-2-one respectively. To the stirred solution of an equivalent amount of 5-methyl-[1,2,4]triazolo[1,5c]quinazolin-2-one and appropriated benzaldehyde in ethanol was added aqueous NaOH solution(10% w/v, 10ml). The title compounds were obtained by using formamide and different secondary amines following mannich reaction (scheme 1).

The structures of synthesized compounds were confirmed by IR, NMR and mass spectral analysis and analytical data of synthesized compounds were shown in Table 1.

Table 1: Analytical data of synthesized compounds

S.No.	Comp.	Structure	Mol.Formula	Mol.wt	m.p. ^{0}C	% yield
1.	**K 11**		$C_{22}H_{22}BrN_5O$	452.35	180	60
2.	**K 12**		$C_{30}H_{22}BrN_5O$	548.43	180	50
3.	**K 13**		$C_{22}H_{21}BrN_6O$	465.35	210	40
4.	**K 14**		$C_{30}H_{22}N_7O_3$	514.53	260	30
5.	**K 15**		$C_{22}H_{21}N_7O_3$	431.35	220	34

6.	**K 16**		$C_{22}H_{22}N_6O_3$	418.45	210	28
7.	**K 17**		$C_{22}H_{22}N_6O_3$	418.45	210	40
8.	**K 18**		$C_{22}H_{23}N_5O_2$	373.45	180	35
9.	**K 19**		$C_{22}H_{22}N_6O_2$	402.45	200	35

5-[2-(4-bromo-phenyl)-vinyl-3-diethylaminomethyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 11)
IR (cm^{-1}): 3383.86 (NH Str), 3041.10 (CH_3 Str), 1383.69 (CO Str), 1942.95 (CH_2 bending), 1593 (NH bending), 500 (Br group). **^{1}H-NMR (δ ppm):** 7.9-6.7 (m, 18H Ar-H), 5.4-5.0 (2H, d, =C=CH), 3.4-3.3 (s, 2H, CH_2). **TOF MS *m/z*:** 461(M^+), 269, 187, 105, 335.

5-[2-(4-bromo-phenyl)-vinyl-3-diphenylaminomethyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one(K 12)
IR (cm^{-1}): 3459.93 (NH Str), 2359 (CN Str), 1590.43 (CO Str), 1020.09 (CH_2 bending), 1590 (NH bending), 590.04 (Br group). **^{1}H-NMR (δ ppm):** 8.6-8.06 (m, 8H Ar-H), 3.3-3.2 (s, 2H, CH_2), 2.56-2.54 (m, 2H, CH_2). **TOF MS *m/z*:** 547.3(M^+), 335.1, 168, 170, 475.

5-[2-(4-bromo-phenyl)-vinyl-3-piperazin-1-yl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 13)
IR (cm^{-1}): 3454.01 (NH Str), 2340.82 (CN Str), 2959.5 (CH_3 Str), 1347.8 (CO Str), 1412.61 (CH_2 bending), 1593.79 (NH bending). **^{1}H-NMR (δ ppm):** 7.9-6.7 (m, 18H Ar-H), 5.4-5.0 (d, 2H, -C=CH_2), 3.4-3.3 (s, 2H, CH_2). **TOF MS *m/z*:** 463(M^+), 105, 269, 346, 187.

3-[(diphenylamino)-methyl]-5-[2-(4-nitro-phenyl)-vinyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 14)
IR (cm^{-1}): 3406 (NH Str), 3041.6 (CH_3 Str), 1706 (CN bending), 1347.8 (CO Str), 1465.61 (CH_2 bending), 1596.79 (NH bending), 1343 (NO_2 group). **^{1}H-NMR (δ ppm):** 8.2-6.7 (m, 18H Ar-H), 5.6-4.9 (d, 2H, -C=CH_2), 2.64-2.0 (s, 2H, CH_2). **TOF MS *m/z*: 513**(M^+), 269, 105, 335, 187.

5-[2-(4-nitro-phenyl)-vinyl-3-piperazin-1-yl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 15)
IR (cm^{-1}): 3406 (NH Str), 2959.67 (CH_3 Str), 1254.71 (CN bending), 1347.8 (CO Str), 1254.61 (CH_2 bending), 1494.1 (NH bending), 1412 (NO_2 group). **^{1}H-NMR (δ ppm):** 8.29-7.0 (m, 8H Ar-H), 6.9-6.7 (d, 2H, -C=CH_2), 3.3 (s, 2H, CH_2), 2.7-2.1 (m, 4H, -N-C). **TOF MS *m/z*:** 430.6(M^+), 220, 170, 336.

3-[(diethylamino)-methyl]-5-[2-(4-nitro-phenyl)-vinyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 16)
IR (cm^{-1}): 3406 (NH Str), 2959.67 (CH_3 Str), 2341.25 (CN Str), 1347.8 (CO Str), 1254.71 (CH_2 bending), 1484.1 (NH bending), 1314(NO_2 group). **^{1}H-NMR (δ ppm):** 8.14-7.56 (m, 8H, Ar-H), 5.6 (d, 2H, -C=CH_2), 2.40 (s, 2H, CH_2). **TOF MS *m/z*:** 419(M^+), 105, 269, 187.

5-[2-(2,4-dihydroxy-phenyl)-vinyl-3-piperazin-1-yl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 17)
IR (cm^{-1}): (3789.47 (OH Str), 2935.51 (CH_3 Str), 2341.71 (CN Str), 1097.59 (CO Str), 1412.44 (OH bending). **^{1}H-NMR (δ ppm):** 8.0 (m, 7H Ar-H), 3.3 (s, 2H, CH_2), 2.5-2.1 (m, 4H, -N-C). **TOF MS *m/z*:** 346(M^+), 269, 187.

3-[(diethylamino)-methyl]-5-styryl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 18)
IR (cm^{-1}): 2935.51 (CH_3 Str), 2341.71 (CN Str), 1097.59 (CO Str). **^{1}H-NMR (δ ppm):** 8.6-8.0 (s, 1H, NH), 7.9-7.0 (m, 9H, Ar-H), 3.6(2H, d, -C=CH_2). **TOF MS *m/z*:** 372(M^+), 170, 182, 187.

5-[2-(4-hydroxy-phenyl)-vinyl]-3-piperazin-1-ylmethyl-[1,2,4]triazolo[1,5-c]quinazolin-2-one (K 19)
IR (cm^{-1}): (3776.09 (OH Str), 3423.88 (NH Str), 2935.51 (CH_3 Str), 2365.30 (CN Str), 1412.69 (OH bending). **^{1}H-NMR (δ ppm):** 8.6-7.2 (m, 8H Ar-H), 6.9-6.8 (d, 2H, -C=CH_2), 2.7-2.3 (m, 4H,$NC_)$, 2.38-2.0 (s, 1H, NH), 2.0-1.9 (s, 2H, CH_2). **TOF MS *m/z*:** 401(M^+), 281, 161, 229, 263.

Methods for the determination of Anti-tubercular activity:
Agar Micro Dilution Method: Drug susceptibility and determination of MIC of the test compounds and standard drugs were performed by agar micro dilution method where serial twofold dilutions of each test compound were added into 7H10 agar and *M. tuberculosis H37Rv* was used as test organism. MIC is the concentration of the compound that completely inhibits the growth and colony forming ability of *M. tuberculosis*. In 24 well plates, 3 mL middle brook 7H11 agar medium with OADC supplement is dispensed in each well. The test compound is added to the middle brook medium agar before in duplicate so that final concentration of test compound in each well is 10, 20 30 µg/mL respectively. The known CFU of *H37Rv* culture was dispensed on top of agar in each well in negative pressure biosafety hood. The plates are then incubated at 37°C/5% CO_2 incubator. The concentration at which complete inhibition of colonies was observed was taken as MIC of test drug and results were shown in Table 2.

Table 2-In vitro antitubercular activity of the synthesized compounds against *M. tuberculosis H37Rv*

S. No.	Synthesized Compound	MIC (µg/ml)	Sensitivity
1.	**K 11**	30	+
2.	**K 12**	30	Nil
3.	**K 13**	30	+++
4.	**K 14**	30	+
5.	**K 15**	30	++
6.	**K 16**	30	+
7.	**K 17**	30	+++
8.	**K 18**	30	+
9.	**K 19**	30	++
Standard	**Streptomycin and Pyrazinamide**	7.5	++++

+= moderate, ++= Good, +++=very good, ++++= excellent

CONCLUSION

All synthesized compounds (K 11-19) resulted in good yields with 50-60%. The anti-tubercular activity of synthesized compounds was performed against M. tuberculosis H37Rv at concentration 30 µg/ml using Streptomycin and Pyrazinamide 7.5 µg/ml as standard drug.

All synthesized compounds have shown good anti-tubercular activity as compared to standard drug Streptomycin, Pyrazinamide. Compounds **K 13** and **K 17** have shown very good anti-tubercular activity. These results reveals that the compounds containing piperazine substitution at 3rd position of Triazolo[1,2,4]quinazolinone nucleus enhances their anti-tubercular activities.

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