INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

SYNTHESIS OF SOME NOVEL 4, 6-DISUBSTITUTED DERIVATIVES AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

Sucheta Gautam[1]*, Dharmendra Mishra[2], Ranjit Singh[2] and DK Pal[1]

[1]College of Pharmacy, Institute of Foreign Trade and Management (IFTM), Lodhipur Rajput, Delhi Road, Moradabad, Uttar Pradesh, India.
[2]Shobhit University, NH-58, Modipuram, Meerut, Uttar Pradesh, India.

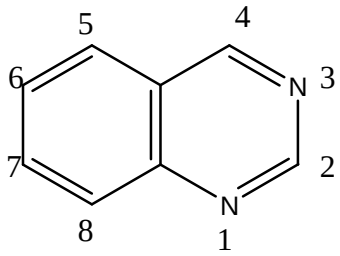
ABSTRACT

In view of the link between use of NSAIDs and altered antimicrobial agents and a growing evidence of COX-II implications in angiogenesis, a novel series of 4, 6-substituted quinazoline derivatives have been synthesized starting from anthranilic acid derivatives through conventional methods. Initially acylation followed by cyclisation to obtain benz-oxazinones which on further treatment with ammonia yielded the crucial intermediate, 2-substituted benzamide. The products were subsequently cyclised to obtain quinazolones, chlorinated, then hooked to have various 4, 6-disubstituted quinazoline derivatives. All the derivatives are screened for anti-microbial activity.

Keywords: Anthranillic acid; Angiogenesis; Amination; Chlorination.

1. INTRODUCTION

Quinazoline (1, 3- diazanaphthalene) was prepared by Gabriel in 1903 although the first derivative was synthesized by Griess. The name was proposed by Widdege, other names such as phenmiazine, benzo-1, 3-diazine and 5; 6-benzopyrimidine has occasionally been used. The numbering suggested by Paal and Busch is still in use.



The presence of a fused benzene ring alters the properties of the pyrimidine ring considerably. The two nitrogen atoms are not equivalent and the marked polarization of the 3, 4- double bond is reflected in the reactions of quinazoline. The properties of substituted quinazolines largely depend on

(a) The nature of the substituent.
(b) Wheather they are in the pyrimidine ring or in the benzene ring,
(c) and whether or not complete conjugation is present in the pyrimidine ring.

The reduction of quinazoline was complicated by covalent hydration in acidic solution, because the hydrated species were not easily reduced. The anhydrous species in alkaline medium were reduced stepwise to dihydro and then to tetrahydroquinazoline and the dihydro radical intermediate was capable of dimerization.The protonation rates of N-heterocyclic in aqueous solution could be determined by polarographic technique. The rates for quinazoline and pyrimidine were too fast for measurement which was consistent with predictions from quantum chemical calculation.

Quinazoline shows broad variety of biological activity profiles, such as analgesic, anti-inflammatory, antibacterial, diuretic, antihypertensive, antimalarial, sedative, hypoglycemic, antibiotic, antitumor etc. These examples clearly demonstrate the potential of quinazoline derivatives as a source of useful pharmacophore for new drug evolution. As our interest in search for biological heterocycles, we sought an unexplored, synthetically accessible heterocyclic template (quinazoline) capable of bearing some potential pharmacophore to elicit and enhance inherent biological activity. In addition, quinazoline derivatives also have a therapeutic benefit as an anti-invasive agent with potential for activity in early and advanced solid tumors, metastatic bone disease and leukemia. Some of the known quinazoline derivatives exhibited remarkable anticancer activity. However search is continuously on to identify more potent lead molecules as these molecules are developing resistance over a period.

Based on the importance of these molecules, our attention was attracted towards synthesis of novel quinazoline derivatives in order to find more potent molecules. Among a wide variety of nitrogen heterocyclic that has been explored for developing pharmaceutically important molecules, the quinazoline have played an important role in the medicinal chemistry and subsequently emerged as a pharmacophore. There has been an increasing interest in the chemistry of 4(3H)-quinazoline because of their biological significance.

The rapid rise in bacterial resistance to the traditional antibiotics such as penicillin and tetracycline has encouraged as a search for new classes of compounds with novel modes of antibacterial activity, the quinazoline nucleus have emerged as an area of immense interest because of their broad spectrum of in *vitro* and there in *vivo* chemotherapeutic efficiency.

2. MATERIALS AND METHODS

Anthranillic acid (2-amino benzoic acid) was reacted with benzoyl chloride in dry pyridine at 0-4°C for 4hrs and obtained benz-oxazinone in high yields. Benz-oxazinone is further treated with aq. Ammonia at room for 30 minutes and results in respective amide derivative. This amide derivative on refluxing with aq.sodium hydroxide gave cyclised product quinazoline-4-one.It is further chlorinated using POCl3/PCl5 and obtained 4-chloroquinazoline.In order to see the role of substituent on rate of reaction, yield of products and subsequently on activity, anthranilic acid was substituted with bromine/iodine in fifth position and the sequence of reactions is carried out to obtain the respective products are independent of substituent used. The sequences of reactions are drawn in scheme and yield of products are tabulated in Table.1.

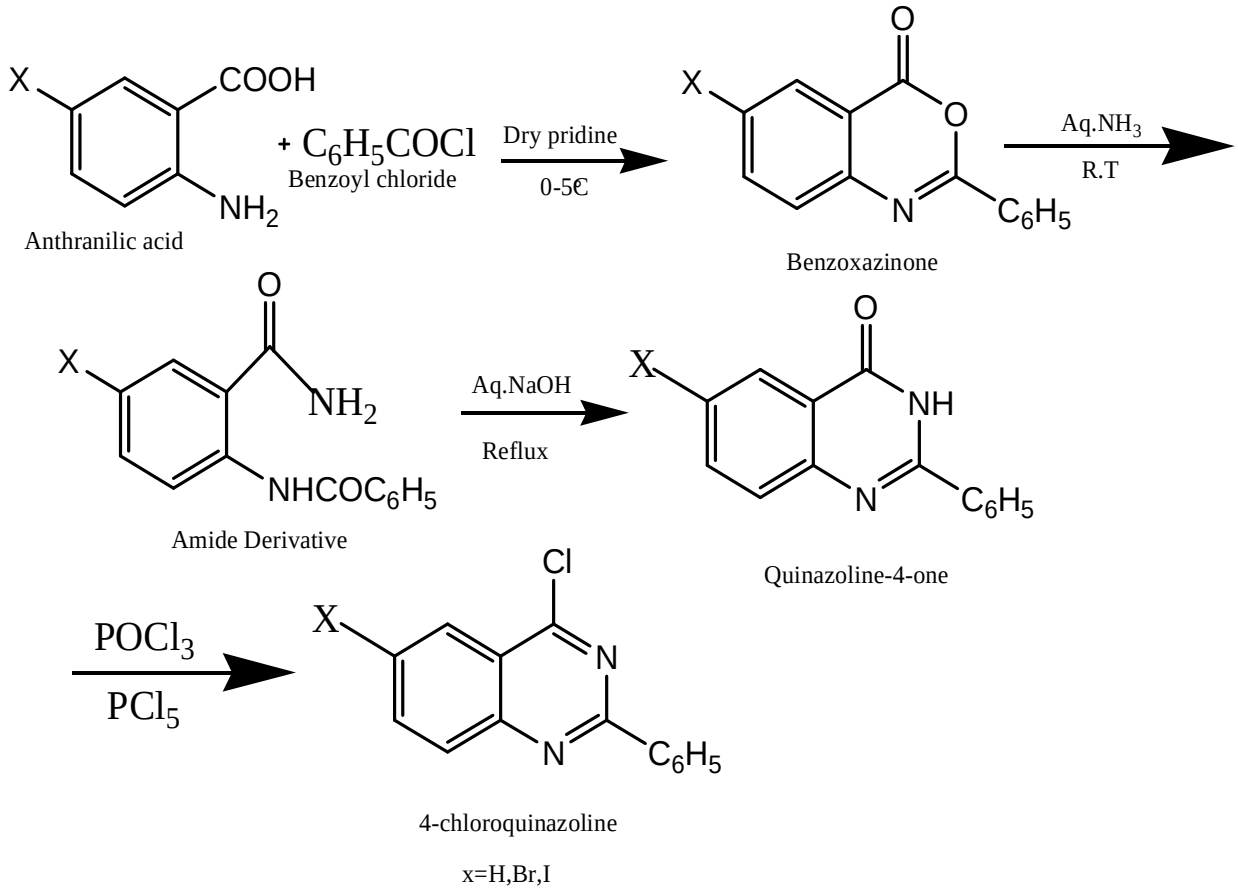


Table 1: Synthesis of 4, 6-disubstituted quinazoline derivatives

Compound no.	X	M.p (°c)	Yield (%)
QZ-1	CH3	95° C	57.3
QZ-2	HBr	100°C	68.2
QZ-3	CH3I	115°C	58.2
QZ-4	OCH3	100°C	69.2

3. Pharmacology

Quinazoline derivatives being considered as potent antimicrobial agents, several new quinazoline derivatives QZ-1 to QZ-4 were synthesized and screened for antimicrobial activities. On the basis of antimicrobial activity it was found that all the synthesized compounds shows good activity against gram positive and gram negative bacteria and antifungal activity against fungi (*Aspergillus fumigatus*).The study showed the structural activity relationship between the antimicrobial activity and certain structural modifications of these new quinazoline derivatives.

3.2 Antimicrobial activity

3.2.1 Inhibition of *Escherichia coli*

All compounds (QZ-1 to QZ-4) showed minimum inhibitory concentration of *E.coli* ranges between 50 to 100 µg/ml. The compound 6-bromo-2-phenylquinazoline-4(3H)-one (QZ-2) showed best activity (MIC, 18µg/ml) among all quinazoline derivatives but showed less activity than that of standard drug.

3.2.2 Inhibition of *Aspergillus Fumigatus*

Compound QZ-2 showed inhibitory effect at concentration 50µg/ml (minimum MIC) and compound QZ-1, QZ-3 and QZ-4 showed inhibitory effect at concentration 100µg/ml respectively.

On the basis of the antimicrobial activity, it was found that the compound QZ-2 showed good antimicrobial activity in comparison to all other synthesized compounds. All synthesized compounds showed antimicrobial activity against *gram positive* and *gram negative* bacteria and antifungal activity against fungi (*Aspergillus fumigatus).* The study showed the structural activity relationship between the antimicrobial activity and certain structural modifications of these new quinazoline derivatives.

The compound QZ-2 was found to be most effective against *Escherichia coli* and *Aspergillus fumigatus* at concentration of 50 and 100µg/ml respectively. The compounds QZ-1, QZ-3 and QZ-4 were found to be least effective against *E.coli* and *A. fumigatus*. In general the order of an antimicrobial activity of quinazolines derivatives is as follows -:

QZ-2> QZ-1> QZ-3>QZ-4

These variations in the antimicrobial activity occurred due to some structural changes in the synthesized compounds. An introduction of substituent at C-6 position in quinazoline nucleus was found to be active against all microorganisms.

Antifungal activity of substituted quinazoline derivative

S. No.	Compounds	Zone of Inhibition (mm)	
		50	100
1.	Qz-1	16	15
2.	Qz-2	16	18
3.	Qz-3	15	14
4.	Qz-4	10	13
standard	Ciprofloxacin	12	14

4. CONCLUSION

A series of novel quinazoline derivatives have been synthesized through a facile strategy and screened for antimicrobial activity. Promising compounds which showed activity have been identified.

5. EXPERIMENTAL SECTION

Melting points were recorded by open capillary method (Thieles tube method) and are corrected.I.R spectra were recorded on a Perkin-Elmer spectrum RXI FT-IR system using KBr optics. 1H NMR Spectra were recorded on Bruker,Advance II 300 MHz and Unity 400 MHz spectrometer in DMSO-d6 or CDCl3 using TMS as an internal standard from SAIF department Punjab University , Chandigarh.Electron impact (EI) and chemical ionization mass spectra were recorded through model NO. Q-T of Micromass spectrometer data system using electron spray ionization from SAIF department Punjab University, Chandigarh.All reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F 254 (mesh); spots were visualized under UV light. Meck silica gel (100-200 mesh) was used for chromatography.

6. GENERAL PROCEDURE

Anthranillic acid (2-amino benzoic acid) was reacted with benzoyl chloride in the presence of dry pyridine at 0-5°C for 4 hrs to obtained benzoxazinone which on further treatment with aqueous ammonia at room temperature for 30 minutes results in amide derivative. The amide derivative on reflux with sodium hydroxide for 4-5 hrs gave the cyclized product quinazoline-4-one.The solid obtained was filtered, washed with water, dried under high vacuum and recrystallized with ethanol.

6.1 6-chloro -2-phenylquinazoline-4(3H)-one -: (Qz-1).

Yield: 0.47g (81.33%); m.p 95C; IR (KBr, cm-1): 1640-3400 (OH, NH); Mol.formula. $C_{14}H_9ClN_2O$

6.2 6-bromo-2-phenylquinazolin-4(3H)-one -: (Qz-2)

Yield: 47.3g (68.2%); m.p 95°C; IR (KBr, cm-1):600-500 (C-Br), 1600-1690 (C=O), 1340-1250
(CN), 1650-1550 (NH), Mol.formula. $C_{14}H_9BrN_2O$

6.3 6-Iodo-2-phenylquinazoline-4(3H)-one-: (Qz-3)

0.71 gm (58.2%); m.p 115°C; IR (KBr, cm-1):600-500 (C-I), 1690-1600(C=O); 1650-1550 (NH); 1690-1600 (C-C); 1340-1250 (CN); 3050-3010(Ar-CH); 1600(C=CAr). Mol.formula.$C_{14}H_9IN_2O$

6.4 6-Methoxy-2-phenylquinazoline-4(3H)-one –: (Qz-4)

0.82gm (69.2%); m.p 100°C; IR (KBr, cm-1): 900-600(Ar-CH), 1040(C-O); 1350-1250(Ar C-N); 1650-1550(NH); 1410-1310 (C=O); 1680-1600(C-C); 3000-2840(CH).Mol.formula.$C_{15}H_{12}N_2O_2$

7. PHARMACOLOGY

7.1 Determination of antibacterial activity

The *in-vitro* antibacterial activity was tested by the disk diffusion method using pathogenic strains of *E.coli.* The concentration 30g/disc of compounds was impregnated on the discs. These discs were placed on the surface of the agar plates already incubated with pathogenic bacteria. The plates were incubated at 37 °C and examined at 48 hrs for zone of inhibition. Ciprofloxacin was used in the assay as a standard control drug. An additional control disc without any sample but impregnated with an equivalent amount of solvent (DMSO) was also used in the assay. The result of antibacterial activity indicated that some of the compounds exhibited mild to moderate activity.

Disc diffusion Assay

The disc diffusion assay was performed in radiation sterilized Petri plates of 10.0 cm diameter. Different concentrations in the range of 750-1.46 g of the test compounds were impregnated on the sterilized discs (5.0 mm in diameter) of Whatman filter paper. The discs were placed on the surface of the agar plates already inoculated with bacterial spores. The plates were inoculated at 37 °C and examined at 24 and 48 hrs for zone of inhibition of at least 6.0 mm diameter, was considered as minimum inhibitory concentration (MIC).

Antibacterial activity of substituted quinazoline derivatives

S. No.	Compound	Zone of Inhibition (mm)	
		50	100
1.	Qz-1	16	15
2.	Qz-2	16	18
3.	Qz-3	15	14
4.	Qz-4	10	13
standard	Ciprofloxacin	29	30

ACKNOWLEDGEMENTS

Authors are thankful to Director Dr. A.K.Ghosh College of pharmacy, IFTM , Staff of national information centre for drugs and pharmaceuticals (NICDAP), centre drug research institute library for the entiring services by them. SAIF department Chandigarh university Punjab. Heartly thankful to all the faculty members of school of pharmaceutical sciences, Shobhit university meerut.

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