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Designing and synthesis of some β-lactam- quinazolone compounds for studying their activity against *Escherichia coli* and *Bascillus subtilis*

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Abstract- The synthesis of 2-Phenyl-3, 1-benzoxazine-4-one 1 have been obtained from the condensation of Antharanilic acid, acid chloride in pyridine.The compound 1 was then converted to its respective quinazolone by condensing with benzidene or Hydrazina hydrates 2or 3. Compound 2 or 3 on heating with aromatic aldehyde gave 3-(p-Arylidenoamino diphenyl)/Arylideno-amino-2-phenyl-4-(3H) quinazolone 4 or 5. Interaction of 4 or 5with chloroacetyl chloride, anhydrous ZnCl2 undergoes cyclization to give 3-(p-Arylidenoamino diphenyl)/2-Phenyl-3-{4 (-aryl)-3-chloro azetidiones)]quinazolone 7a-e and 6a-e. The new compounds have been screened for its bioevaluation against antimicrobial agents.
Keywords- Quanzolone, □-lactam, chloroacetyl chloride, triethylamine, antharanilic acid, 1,1-biphenyl-4,4-diamine

Introduction

Quanzolone are familiar group of heterocyclic compounds possessing a wide verity of antimicrobial activity [1-4] and the derivatives of qunazolone have a therapeutic benefits as an antiinvasive agents with potential activity in early and advanced solid tumors, meta static bone disease and leukemias [5,6]. Where as the □-lactam antibiotics are widely known for there clinically important drugs which have over the year provoked an extraordinary amount of activity by synthetic organic chemist [6-12]. The constant research effort for the designing and development of new chemical candidate molecules has been necessitated. Since these compounds contain two main structural features in their molecular architecture viz., quanzolone and □-lactam.

Antimicrobial Screening

Compounds 6a-e and 7a-e were screened for their antibacterial activity against *Escherichia coli* and *Basillus subtilis in vitro* involving the two fold serial dilution technique as recommended by the National Committee for Clinical Laboratory Standards(NCCLS) [13]. The activity data for all compounds tested is presented in table-II. Antimicrobial activity data for compounds 6a-e are suggestive of unsuitability of such compounds as for as against highly pathogenic strain of *E. coli* is concerned since only one compound was found to show a marginal degree of activity. Out of five compounds only one compound 6a having a p-Cl substituents was found to exhibit very low order of antimicrobial activity since MIC obtained was 50. Antimicrobial data of compounds 7a-e against *B. subtilis* are also suggestive of either very weak activity or non existent activity of such compounds since only two compounds showed very low order of antimicrobial activity. Here, both the compounds bearing hydroxyl function at ortho and para position displayed the same order of activity. Thus, the compound no. 7a had MIC value of 12.5 µg/ml while for other three compounds the MIC values are very high. It is interesting to observe here that an electronegative group present (R=p-Cl) at the para position seems mainly responsible for causing a change in the activity since for o-OH substituted compound the MIC obtained was >100.There may be two ways to explain these results. First, p-Cl substituted compound is able to penetrate the cell wall of E. coli thus preventing the biosynthesis of the polymer peptidoglycan or secondly it is finding better fit at the receptor site as compound to other three compounds. On evaluation for their antimicrobial activity against *B. subtilis* compound 7a-d furnished some very valuable results. Two of the four compounds of this category were found to inhibit the growth of bacteria to a considerable extent while two other were found least significant. One compound 7d is highly active. This compound bearing a 4-OH, 3-OCH3-Phenyl has displayed a comparable activity since MIC was found to be 6.25 µg/ml while compound 7a showed a comparatively diminished activity (MIC 12.5µg/ml). These results can be interpreted in terms of electronic factors. Nitro group has a –I and +R effect from these position. However more study is required involving the synthesis of additional compounds bearing more electronegative group and subjecting these new chemical candidate molecules for their in vitro and in vivo assessment against same and other bacterial strain.

Experimental procedure

All the melting points were uncorrected and recorded on cinten melting point apparatus. IR spectra were recorded in KBr on a Perkin-Elmer 337 spectrophotometer. 1H-NMR spectra on a varian Gemini 200 MHz Spectrometer using TMS as internal standard (chemical shift values are expressed in δppm) and mass spectra on a micromass instrument operating at 70 eV.

2-Phenyl-3, 1-benzoxazine-4-one 1

Antharanilic acid (0.2 mole) was dissolved in dry pyridine (100 ml) by stirring at room temperature. The solution was cooled to 0oC and acid chloride (0.4 mole) was added slowly with constant stirring. When the addition was complete, the resultant reaction mixture was stirred for half an hour at room temperature. It was treated with 10% NaHCO3 solution in order to dissolve any unreacted acid. The solid thus obtained, was washed repeatedly with water and air dried. The crude benzoxazine thus obtained, was recrystallized from diluted ethanol as white crystalline mass m.p. 124oC13, Yield 85% [14].

3-(p-Amino diphenyl/2-Phenyl-3-amino-4-(3H)-quinazolone 2 (2&3)

Compound 2 was synthesized in the following manner. A mixture of 2-Phenyl-3,1-benzoxazine-4-one (0.05 mole) and 1,1-biphenyl-4,4-diamine (0.05 mole) in anhydrous pyridine (50 ml) was heated under reflux on a sand bath for 6 hours under anhydrous conditions. Subsequently, the reaction mixture was poured into ice cold water (100 ml) containing conc. HCl (10 ml). A solid started to separate out, which was allowed to settle down for 1 hour. It was filtered off and washed sucessessively with water. After drying in vacuum, it was recrystallized from ethanol. m.p. 185oC, yield 75%. Analysis for C25H19N3 N: Calculated: 18.66, Found: 18.59. IR(KBr): 3442 (N – H str), 3055 (Ar-H), 1733 (C=O), 1648 (C=N), 1611-1421 (C=C skeletal);

Compound 3 was synthesized in the following manner. A mixture of 2-phenyl-3,1-benzoxazine-4-one (0.2 mole) and hydrazine hydrate in anhydrous pyridine (30 ml) was heated under reflux for 6 hours under anhydrous reaction condition. Subsequently, the reaction mixture was poured into ice-cold water (100 ml) containing concentrated HCl (10 ml). A solid started to separate out, which was allowed to settle down for 1 hour. It was filtered off and washed successively with water. After drying under vacuum it was recrystallized from diluted ethanol. It melted at 166oC5, yield 78%. Analysis for C13H11N3O N: Calculated:11.14, Found: 11.11. . IR(KBr): 3451 (N – H str), 3045 (Ar-H), 1723 (C=O), 1655 (C=N), 1610-1420 (C=C skeletal);

3-(p-Arylidenoamino diphenyl)/Arylideno-amino-2-phenyl-4-(3H) quinazolone (4a-e & 5a-e)

A mixture of 3-(p-Arylidenoamino diphenyl)/2-Phenyl-3-arylidene-amino-4-(3H) quinazolone (0.02 mole) and (0.02 mole) of an appropriate aryaldehyde in absolute EtOH (30 ml) in presence of glacial acetic acid (1 ml) was refluxed for 8-10 hours. Excess of solvent was removed under reduced pressure. The solid obtained, was washed with cold water. Several times and recrystallized from methanol. Characterization data of the compounds thus synthesized, are given in Table-I.

4a: IR(KBr): 2916 (Ar-H), 2321 (N-N), 1634(ter. amide C=O), 1620 (C=N), 1605-1410 (C=C skeletal), 781 (Ar-Cl);

4b: 3395 (Ar-OH), 3052 (Ar-H), 1632-1424 (C=C skeletal), 1620 (C=N), 1616 (ter.amide C=O);

4c: 1621 (C=N), 3356 (Ar-OH), 1648 (tert. amide C=O), 1640 (C=C skeletal), 2366 (N-N), 2933 (Ar-C-H-str.); 1H NMR (CDCl3): □: 4.72 (s, 1H, N-CH-R), 6.76-7.89(m, 21H, Ar-H),

4d: 3011 (Ar-H), 2988 (C-H str), 2341(N-N), 1668 (C=N), 1636 (ter. amide);

4e: 3038 (Ar-H), 1640-1431 (C=C skeletal), 1650 (ter.amide C=O)

5a: IR(KBr): 2355 (N-N), 1635 (ter. Amide C=O), 1622(C=N), 1692-1435 (C=C skeletal), 785 (Ar-Cl);

5b: 3412 (Ar-OH), 2340 (N-N), 1631 (ter. Amide C=O), 1622 (C=N), 1590-1440 (C=C skeletal);

5c: 1631 (C=N), 3345 (Ar-OH), 2366 (N-N), 2941 (Ar-CH-str.), 1650(tert. amide C=O), 1652 (C=C skeletal), 1H NMR (CDCl3): □ 4.51 (s, 1H, -N-CH-R), 6.42-7.78(m, 12H, Ar-H), 5.32 (s, 1H, Ar-OH);

5d: 1651 (C=N), 1641 (ter. Amide C=O), 1634-1471 (C=C skeletal)

3-(p-Arylidenoamino diphenyl)/2-Phenyl-3-{4 (-aryl)-3-chloro azetidiones)] quinazolone (6a-e& 7a-e)

A mixture of compound 4 or 5 (0.01 mole), in dixon (50 ml) were added chloroacetyl chloride (0.01 mole) and triethylamine (0.01 mole) at 0 C with stirring. The reaction mixture was left at room temperature for 3 hours and then refluxed for 10 hours. Excess of solvent was distilled off and the residue was poured into crushed ice and recrystallized from diluted ethanol. The compounds thus synthesized are incorporated in table –I.

6a. IR(KBr): 3033 (Ar-H), 1691 (C=O), 1641 (C=N), 3419(N-H), 1722 (C=O),1181(C-O str); 1HNMR(CDCl3)□: 7.15-7.71(m,21H,Ar-H), 8.30(brs,1H,CONH), 3.64(d,1H,Cl-CH), 3.91(d,1H,N-CH-R), 5.15(s,1H,Ar-OH);

6a: Mass (FAB): 435 (M+), 419, 217, 201, 145, 119, 111, 105 (Base peak);

6b. IR(KBr): 3039(Ar-H), 1681 (C=O), 1635 (C=N), 3419(N-H), 1727 (C=O),1189(C-O str); 1HNMR(CDCl3)□: 7.21-7.77(m,21H,Ar-H), 8.36(brs,1H,CONH), 3.61(d,1H,Cl-CH), 3.90(d,1H,N-CH-R), 5.18(s,1H,Ar-OH);

6e: Mass (FAB): 447 (M+), 419, 217, 201, 145, 119, 111, 105 (Base peak);

7a. IR(KBr):1679(See.amide C=O), 1658(tert, amide C=O), 723(C-Cl), 1628(C=N), 3381(N-H), 2978(C-H); 7c. 1HNMR(CDCl3) □: 7.22-7.75 (m,13H,Ar_H), 4.25(d,1HCl-CH), 8.41(brs,1H,CONH) ;

7a: Mass (FAB): 587(M+), 553, 494, 477, 362, 285,220, 198,145, 119,105(Base peak);
7b. IR(KBr):1681(See.amide C=O), 1666(tert, amide C=O), 715(C-Cl), 1632(C=N), 3385(N-H), 2985(C-H); 7c. 1HNMR(CDCl3) □: 3.32(s,3H,Ar-OCH3), 4.95(s,1H,Ar-OH), 7.22-7.81(m,13H,Ar_H), 4.21(d,1HCl-CH);
7b: Mass (FAB): 568(M+), 551, 447, 477, 361, 285,220, 198,180,145, 123,119,105(Base peak);

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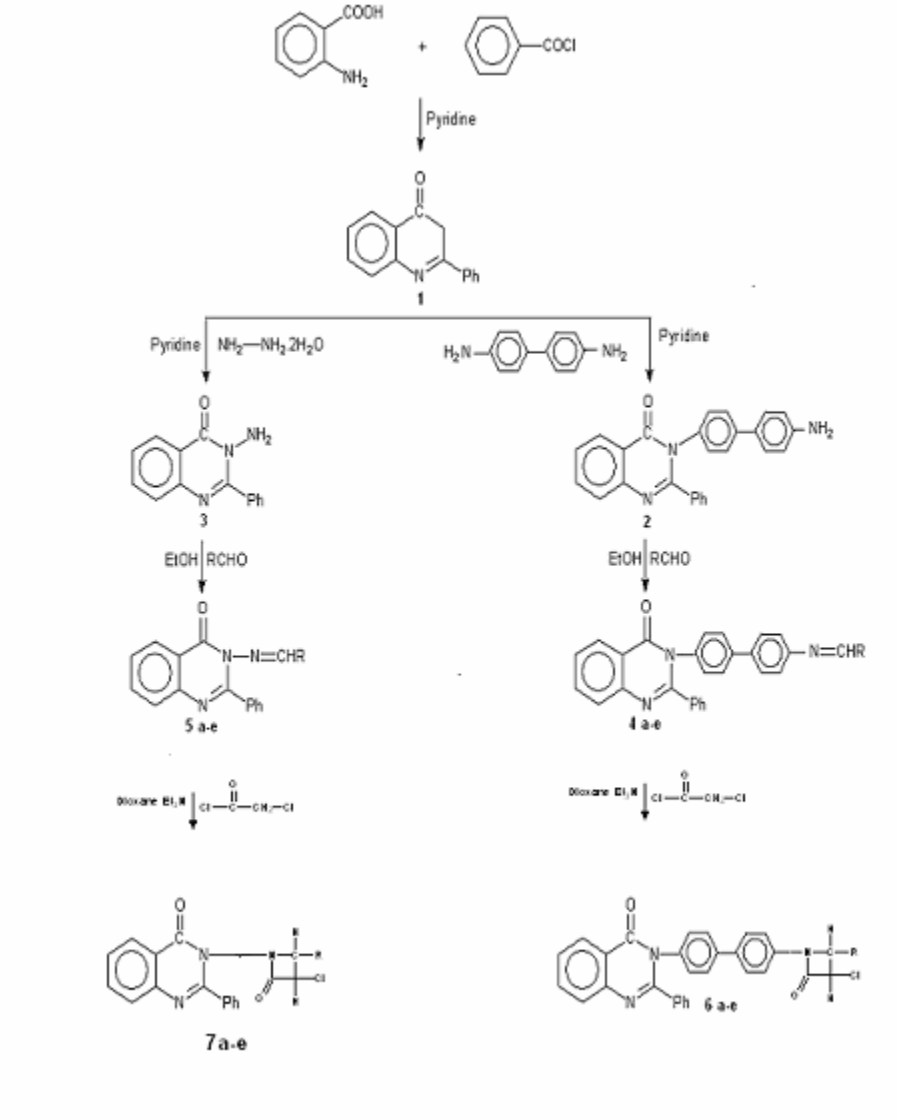
Table I- Characterization data for Compounds 4a-e, 5a-d, 6a-e & 7a-d

R	Compd.	m.p. ^{o}C	Yield (%)	Mole. formula	Analysis (%) nitrogen (Calcd) Found
p-chlorophenyl	4a	158	60	$C_{33}H_{22}N_3O_2Cl$	7.91 (7.96)
	5a	171	85	$C_{21}H_{14}N_3OCl$	11.12 (1168)
	6a	175	80	$C_{35}H_{23}N_3O_2Cl_2$	7.11 (7.14)
	7a	98	80	$C_{23}H_{15}N_3O_2SCl_2$	9.58 (9.63)
o-Hydroxyphenyl	4b	210	75	$C_{33}H_{23}N_3O_2$	8.47 (8.51)
	5b	235	55	$C_{21}H_{15}N_3O_2$	12.37 (12.31)
	6b	120	72	$C_{35}H_{24}N_3O_3Cl$	7.31 (7.37)
	7b	87	70	$C_{23}H_{16}N_3O_3Cl$	9.97 (10.05)
p-Hydroxyphenyl	4c	154	80	$C_{33}H_{23}N_3O_2$	8.56 (8.51)
	5c	217	80	$C_{21}H_{15}N_3O_2$	12.27 (12.31)
	6c	183	60	$C_{35}H_{23}N_3O_3$ Cl	7.31(7.37)
	7c	168	80	$C_{23}H_{16}N_3O_3$ Cl	9.96 (10.05)
3-OH, 4-OCH_3-phenyl	4d	176	65	$C_{34}H_{25}N_3O_3$	8.00 (8.03)
	5d	227	55	$C_{22}H_{17}N_3O_3$	11.24 (11.32)
	6d	182	65	$C_{36}H_{26}N_3O_4$ Cl	6.91 (7.00)
	7d	110	68	$C_{24}H_{18}N_3O_4$ Cl	9.32 (9.38)
4-OH, 3-OCH_3-phenyl	4e	182	50	$C_{34}H_{25}N_3O_3$	7.98 (8.03)
	5e	171	75	$C_{22}H_{17}N_3O_3$	11.21 (11.32)
	6e	182	50	$C_{36}H_{26}N_3O_4$ Cl	7.07(7.00)
	7e	148	50	$C_{24}H_{18}N_3O_4$ Cl	9.47 (9.38)

Table II- Antimicrobial activities of compounds 6a-e & 7a-d

Compd.	R	Minimum inhibitory concentra-ion (MIC) µg/m*l* against E. coli	Compd.	R	Minimum inhibitory concentra-tion (MIC) µg/m*l* against B. subtilis
6a	p-chlorophenyl	50	7a	p-chlorophenyl	6.25
6b	o-Hydroxyphenyl	25	7b	o-Hydroxyphenyl	>100
6c	p-Hydroxyphenyl	12.5	7c	p-Hydroxyphenyl	50
6d	4-OH, 3-OCH_3-Phenyl	50	7d	4-OH, 3-OCH_3-Phenyl	25
6e	3-OH, 4-OCH_3-Phenyl	12.5	7e	3-OH, 4-OCH_3-Phenyl	50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NH2
COCl
Pyridine
O
N
Ph
1
Pyridine
NH2—NH2.2H2O
H2N
NH2
Pyridine
NH2
3
2
EtOH
RCHO
N=CHR
5 a-e
4 a-e
Dioxane Et3N
Cl—C(=O)—CH2—Cl
Cl
7a-e
6 a-e

Scheme – 1