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Synthesis and Evaluation of Some 2-Phenyl-4-(Substituted Phenylamines)-Benzo-1,3-Diazine Derivatives with Antimicrobial Activities

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ABSTRACT

Benzo-1,3-diazine based novel antimicrobial agents were designed, developed with different type of functional groups from substituted aromatic amines. In the current development work 2-amino benzamide/anthranilamide react with benzoyl chloride results in 2-phenyl-4(3H)-quinazolinone (I) which undergo chlorination reaction (Vilsmeier- Haack Reaction / Vilsmeier reaction) in presence of $POCl_3$ converted to 4-chloro-2-phenylbenzo-1,3-diazine (II).The Intermediate4-chloro-2-phenyl quinazoline (II) condensed with different aromatic amines in presence of Isopropyl alcohol (IPA) results in 4-benzamine substituted benzo-1,3-diazine derivatives**.**An efficient pyridine catalyzed approach to quinazolinone derivatives has been developed, and the protocol uses cheap and readily available substituted 2-amino benzamide as the starting materials. This can be the example of constructing benzofusednitrogen containing six membered heterocycles. Structure of all synthesized compounds obtained is supported by spectra and analytical data (IR,H-1 NMR and Mass Spectroscopy). All 4-amino substituted quinazoline derivatives of concentration 50µg/ml using DMSO as solvent against E. coli, Pseudomonas aeruginosa, Staphylococcus aureus& C. albicans with Nystatin& Gentamicin was used as a standard. Compounds QZ2Cl and QZ3,4Cl shown greater zone of inhibition as compared to standard.

KEYWORDS: Benzo-1,3-diazine,Quinazolinone, Vilsmeier- HaackReaction

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INTRODUCTION

Heterocyclic compounds containing nitrogen atoms represent an important group of organic compounds because many of them exhibit significant biological activity.Quinazoline is a heterocyclic double-ring structure compound containing benzene fused to pyrimidine.[1]Quinazolines have drawn much attention because of their wide applications as antimicrobial[2-3] and pharmaceutical activities [4-6].The quinazolinones are considered to be a "privileged structure" for drug development.The chemistry of 4(3H)-quinazolinone has received an increasing interest because of its biological significance. The quinazolinones are classes of fused heterocyclic that are of considerable interest because of the diverse range of their biological properties viz. anticancer, antiinflammatory, anticonvulsant, anti-hypertensive and anti-microbial activities.[7-9]

Quinazolinones is a building block for approximately 120 naturally occurring alkaloids isolated till date foam a number of families of the plant Kingdom, from animals and microorganism. Methaqualone was synthesized in 1951 and knows as sedativehypnotic effects and Metalazone as anti hypertensive.Quinazolina is a bicyclic compound consisting of a pyrimidine system fused at 5,6-position with benzene ring. Quinazoline is known as 1,3- diazonapthalene represented by structure (4). It has also been given names like phenmiazine, benzo-1,3diazino or 5,6-benzo pyrinidine.[10-11]Quinazoline was first prepared by Gabriel in 1903[12]. Quinazoline is a solid, m.p. 48°C and can be recrystallised from ether. It is soluble in water and most organic solvents. It is basic in nature (pka-1.4).[13] Among many derivatives of quinazoline system known so far, an attempt is to synthesize some keto-quinazolines also known as Quinazolinones, are the most important compounds.[14]

BIOLOGICAL ACTIVITY AND ITS IMPORTANCE:-

The 4-(3H) Quinazolinones are an important class of fused heterocyclic with a wide range of biological activities such as, antimicrobial, anti bacterial, antifungal, antiinflammatory, anti-convulsant, anti-hypertensive, anti-malarial, anti-HIV, anti-cancer etc. agents. Quinazolinone derivatives are used in a number of dyes. They are also found applications in industry as sensitizers, co-polymer etc. In Agriculture quinazolinone derivatives are used as Herbicides, Insectisides, Bacteriacides etc.

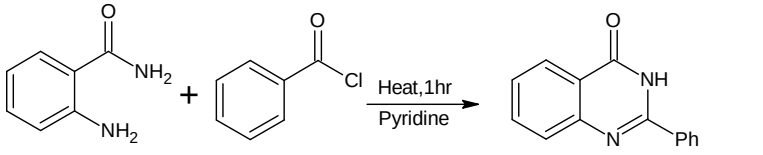
ANTIMICROBIAL ACTIVITY

"Antimicrobial Activity may be defined as activity tending to destroy microbes, prevent their development or inhibit their pathogenic action.[15]Antimicrobial agents is defined as ay compound that selectively destroys or inhibits the growth of microorganism.[16]The antimicrobial activity of quinazolinone and its derivatives which can deal with the increasing resistance by microbes and also having significant antimicrobial activity for the effective treatment of various types of microbial

diseases.[17]Hosakera D. Revanasiddappa et al.' Synthesized a series of Quinazolinone compounds, having antimicrobial activity against the bacteria E.coli and S. aureus and fungi. Aspergillusnigerand A. flavus.[18]4-(3H)-quinazolinone which are most prevalent either as intermediate or as natural products in many proposed biosynthetic pathways.24 This is partly due to the structure being derived from the anthranilates. (Anthralinic acid, anthranilamide and anthranilonitrile).The 4(3H)-Quinazolinones are an important class of fused heterocyclic with a wide range of biological activities such as anti-cancer, anti-inflammatory, anti-malarial, anti-convulsant, and anti-hypertensive etc.

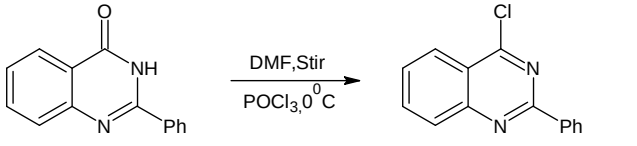
RESULTS AND DISCUSSION:

2-amino benzamide/anthranilamide (1) was treated with distilled benzoyl chloride in presence of dry pyridine/triethylamine (TEA) in slight excess of benzoyl chlorideand reflux for 1.0 hour to obtain 2-phenyl-4(3H)-quinazolinone (I) it involve attack of nucleophile of electrophilic carbon of benzoyl chloride which lead to nucleophilic aromatic substitution type of reaction yields (I),a catalytic amount of pyridine is used i.e pyridine catalysed reaction it is avery clean reaction gives maximum yield of(I)



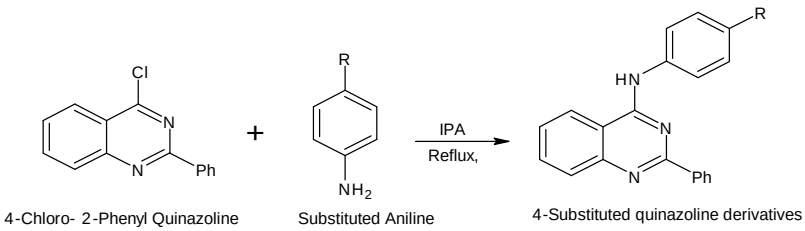
Anthranilamide Benzoyl Chloride 2-phenyl quinazolin-4(3H)-one

Intermediate (I) undergo chlorination reaction (Vilsmeier- Haack Reaction / Vilsmeier reaction) in presence of $POCl_3$ converted to 4-chloro-2-Phenyl benzo-1,3-diazine(II).2 phenyl quinazoline-4-(3H)-one was dissolve in Dimethyl formaide (DMF), addition of $POCl_3$done by drop wise at 0^0C with continuous stirring. After addition of $POCl_3$ stir it at room temperature for 30 min and heat the reaction mixture for 4 hr. at 70^0c.Product was confirmed by TLC, melting point and chemical test.



2-phenyl quinazolin-4(3H)-one 4-Chloro-2-phenyl quinazoline

The Intermediate II condensed with different aromatic amines in presence of Isopropyl alcohol (IPA) results in 4-amino substituted benzo-1,3-diazinederivatives.Isopropyl alcohol (IPA) is very versatile polar solvent involve cleaner reaction with good yield.Electron donating as well as electron withdrawing substituent attached to aniline are condensed with (II) to yield different type of proposedderivatives.



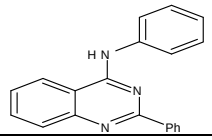
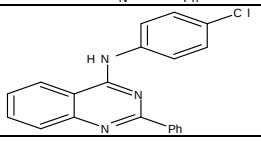
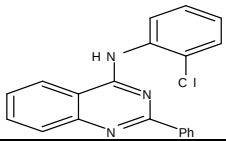
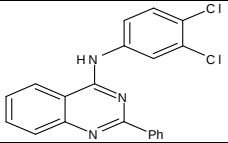
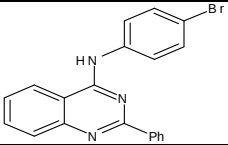
4-Chloro- 2-Phenyl Quinazoline Substituted Aniline 4-Substituted quinazoline derivatives

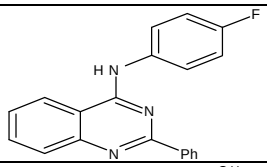
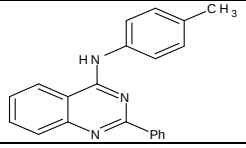
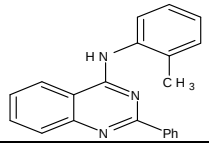
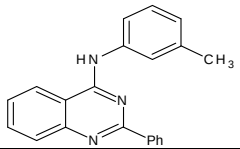
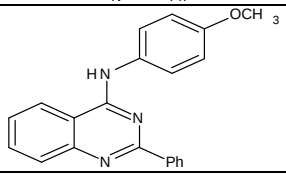
An efficient pyridine catalyzed approach ishas been used to developedquinazolinonederivatives, and the protocol uses cheap and readily available substituted 2-amino benzamide as the starting materials. This can be the example of constructing nitrogen containing six membered benzofusedheterocycles.

Table 1.Physical Characterisation Data For Synthesized Compounds

Starting Material	Reagent used	C-2 Substitution	C-4 Substitution	Molecular Weight	MP (^{0}C)	R_f Value	Yield (%)
2-Amino Benzamide	Benzoyl Chloride	-C_6H_5	Aniline	297	202-204	0.73	62.03
		-C_6H_5	4-Chloro Aniline	330	145-147	0.60	92.45
		-C_6H_5	2-Chloro Aniline	330	182-184	0.73	71.03
		-C_6H_5	3,4-Dichloro Aniline	363	108-110	0.76	82.15
		-C_6H_5	4-Bromo Aniline	375	160-162	0.74	66.66
		-C_6H_5	4-Fluoro Aniline	315	196-198	0.69	83.67
		-C_6H_5	4-Methyl Aniline	311	148-150	0.97	82.50
		-C_6H_5	2-Methyl Aniline	311	190-192	0.73	91.88
		-C_6H_5	3-Methyl Aniline	311	200-202	0.78	71.06
		-C_6H_5	4-Methhoxy Aniline	327	165-167	0.87	55.45

Table 2. Structures For Synthesized Compounds

Code	Structure Of Synthesized Compounds	Molecular Formula
QZAl		$C_{20}H_{15}N_3$
QZ4Cl		$C_{20}H_{14}N_3Cl$
QZ2Cl		$C_{20}H_{14}N_3Cl$
QZ3,4Cl		$C_{20}H_{13}N_3Cl_2$
QZ4Br		$C_{20}H_{14}N_3Br$

QZ4Fl		$C_{20}H_{14}N_3Fl$
QZ4Tl		$C_{21}H_{17}N_3$
QZ2Tl		$C_{21}H_{17}N_3$
QZ3Tl		$C_{21}H_{17}N_3$
QZOTl		$C_{21}H_{17}N_3O$

EXPERIMENTAL METHOD:

Synthesis. The synthetic procedures of synthesizedcompounds are detailed below. These methods are representative for those used for the preparation of other derivatives.

2-phenyl quinazolin-4(3H) (I):

Mix 0.01M anthranilamide and 0.01M benzoyl chloride, catalytic amount of pyridine. Reflux for 1hr.Cool the solution, filter it & wash with water to remove excess of pyridine. Crude product recrystallized from ethanol (Yield: 61.01% M.P: 204-206^{0}C)

ATR 3388.93 cm^{-1} (N-H stretch) ,1662.64 cm^{-1} (N-H bend), 3188.33 cm^{-1} C-H stretch(aromatic), 1714.72 cm^{-1} C=O (amide) , 1255.66 cm^{-1} (C-N stretch) , 1H NMR (500 MHz, $CDCl_3$) δ 3.32-3.33(s, 1H) , δ 7.3-7.63 (8H), HRMS (m/z): [M + H]+, calcd for $C_{14}H_{10}N_2O$, 223 found, 223.10

4-chloro-2-phenyl benzo-1,3-diazine (II):

Compound **(I)** 0.01 M was dissolve in DMF, add a drop wise of 0.02 M $POCl_3$ at 0^{0}C with continuous stirring. After addition of $POCl_3$ stir it at room temperature for 30 min and heat the reaction mixture for 4.0 hrs. at 70^{0}c. Transfer reaction mixture in crush ice yellow precipitate was obtained. Crude product recrystallized from ethanol.

ATR: 3387.00 cm-1 (N-H stretch), 1660.71 cm-1 (N-H bend), 3180.62 cm-1 (C-H stretch (Aromatic)), 1255.66 cm-1 (C-N stretch), 761.88 cm-1 (C- Cl)

2-**phenyl-4-(phenyl amine) benzo-1,3-diazine (III):** 4-Chloro-2-phenyl benzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.2 M of aniline was added. Reaction mixture was refluxed for 10hrs. Reaction mixture was poured in the petri plate. IPA was 4-Chloro-2-phenyl quinazoline-(3H) evaporated to isolate the dried product. Crude product recrystallized from ethanol.

ATR : 3464.15cm^{-1}(N-H stretch), 1616.35 cm^{-1} (N-H bend), 3039.81 cm^{-1} (C-H stretch (Aromatic)), 1288.45cm^{-1} (C-N stretch), 1485.19 cm^{-1} (C=C Stretch Aromatic). 1H NMR (500 MHz, $CDCl_3$), δ 3.61- 3.65 S 1 (H), δ 7.31-7.68 8 (H), HRMS (m/z) : [M+H]+, calcdfor $C_{20}H_{15}N_3$, 297 found 297.82

2-phenyl-4-(3,4-dichlorophenyl amine)benzo-1,3-diazine (IV):

4-Chloro-2-phenyl benzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.2 M 3.4-dichloro aniline was added. Reaction mixture was refluxed for 8.0 hrs. Reaction mixture was poured in the petri plate. IPA was evaporated to isolate the dried product. Crude product recrystallized from ethanol.

ATR: 3410.15cm^{-1} (N-H stretch), 1620.21 cm^{-1} (N-H bend), 3039.81 cm^{-1} (C-H stretch (Aromatic)), 1296.16 cm^{-1} (C-N stretch), 1465.90cm^{-1} (C=C Stretch Aromatic), 694.37 cm^{-1} (C-Cl), 640.37cm^{-1} (C-Cl).1H NMR (500 MHz, $CDCl_3$) δ 3.34 S1(H), δ 7.36-7.61 7(H), δ6.51-6.59 S 1(H), δ 6.51-6.59 S 1(H).HRMS (m/z) : [M+H]+, calcdfor $C_{20}H_{13}N_3C_{l2}$366 found 366.13.

2-phenyl-4-(4-bromophenyl amine)benzo-1,3-diazine (V):4-Chloro-2-phenyl benzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.2 M 4-bromo aniline was added. Reaction mixture was refluxed for 10-12 hrs. Reaction mixture was poured in the petri plate. IPA was evaporated to isolate the dried product. Crude product recrystallized from ethanol.

ATR: 3305.99 cm^{-1} (N-H stretch), 1604.77 cm^{-1} (N-H bend), 3039.81 cm^{-1} (C-H stretch (Aromatic)), 1300.02cm^{-1} (C-N stretch), 1500.62 cm^{-1} (C=C Stretch Aromatic), 586.36 cm^{-1} (C-Br). 1H NMR (500 MHz, $CDCl_3$) δ 3.47 s1(H), δ 7.59 7 H, δ 6.57-6.79 S 1(H), HRMS (m/z) : [M+H]+ $C_{20}H_{14}N_3Br$ 376 found 376.11.

2-phenyl-4-(4-fluorophenyl)amino]benzo-1,3-diazine (VI):

4-Chloro-2-phenyl benzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.2 M 4-fluoro aniline was added. Reaction mixture was refluxed for 9.0 hrs. Reaction mixture was poured in the petri plate. IPA was evaporated to isolate the dried product. Crude product recrystallized from ethanol.

ATR:3170.97cm-1(N-H stretch), 1620.21 cm^{-1} (N-H bend), 3043.67 cm^{-1} (C-H stretch (Aromatic)), 1300.02 cm^{-1} (C-N stretch), 1462.04 cm^{-1} (C=C Stretch Aromatic), 1145.72cm-$^{-1}$ (C-F).

2-**phenyl-4-(4-methyl phenyl amine)benzo-1,3-diazine (VII):**

3- 4-Chloro-2-phenyl benzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.2 M 4-methyl aniline was added. Reaction mixture was refluxed for 10 hrs. Reaction mixture was poured in the petri plate. IPA was evaporated to isolate the dried product. Crude product recrystallized from ethanol.

ATR:3317.56cm^{-1} (N-H stretch), 1620.21 cm^{-1} (N-H bend), 3170.97cm^{-1} (C-H stretch (Aromatic)), 1296.16 cm^{-1} (C-N stretch), 1462.04 cm^{-1} (C=C Stretch Aromatic), 3039.81 cm-1(C-H stretch (CH3)). 1H NMR (500 MHz, $CDCl_3$) δ3.47 S1(H), δ7.31-7.38 8(H),δ0.9-1.0 S3 (H). HRMS (m/z) : [M+H]+ calcdfor $C_{21}H_{17}N_3$311 found 311.19.

2-phenyl-4-(4-chlorophenyl amine)benzo-1,3-diazine (VIII) :

4-Chloro-2-phenyl benzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.2 M of 4-chloro aniline was added. Reaction mixture was refluxed for 8.0 hrs. Reaction mixture was poured in the petri plate. IPA was evaporated to isolate the dried product. Crude product recrystallized from ethanol.

ATR: 3170.97cm-1(N-H stretch), 1620.21cm-1 (N-H bend), 3043.67 cm^{-1} (C-H stretch (Aromatic)), 1300.02 cm^{-1} (C-N stretch), 1462.04cm^{-1} (C=C Stretch Aromatic), 767.67cm-1 (C-Cl)

2-phenyl-4-(2-chlorophenyl amine)benzo-1,3-diazine (IX):

4-Chloro-2-phenyl benzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.2 M of 2-chloro aniline was added. Reaction mixture was refluxed for 10 hrs. Reaction mixture was poured in the petri plate. IPA was evaporated to isolate the dried product. Crude product recrystallized from ethanol.

ATR: 3170.97 cm^{-1} (N-H stretch), 1616.35cm^{-1} (N-H bend), 3035.96cm^{-1} (C-H stretch (Aromatic)), 1292.31 cm^{-1} (C-N stretch), 1462.04cm^{-1} (C=C Stretch Aromatic), 771.53cm^{-1} (C-Cl).

2-phenyl-4-(4-methoxyphenyl amine)benzo-1,3-diazine (X):

4-Chloro-2-phenyl benzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.2 M 3-methyl aniline was added. Reaction mixture was refluxed for 8.0 hrs. Reaction mixture was poured in the petri plate. IPA was evaporated to isolate the dried product. Crude product recrystallized from ethanol.

ATR : 3388.93cm^{-1} (N-H stretch), 1597.06 cm^{-1} (N-H bend), 3188.33 cm^{-1} (C-H stretch (Aromatic)), 1296.16cm^{-1} (C-N stretch), 1450.47 cm^{-1} (C=C Stretch Aromatic), 3062.96 cm^{-1} (C-H stretch (CH3)).

ANTIMICROBIAL ACTIVITY:

This method is based on the principle that antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, pick-up moisture and the antibiotic diffuse radially

outward through the agar medium producing an antibiotic concentration gradient. The concentration of the antibiotic at the edge of the disk is high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth.

Antibacterial Study: Media used- Nutrient Agar media

Strains used-

E. coli ATCC 25922

Pseudomonas aeruginosa ATCC 27853

Staphylococcus aureus ATCC 25923

Candida albicansATCC 10231

Incubation period- 35 to 37˚C for 16 to 18 hours.

Reference compound – Gentamycin and Nystatin

DISC DIFFUSION METHOD-

- The disk diffusion method is performed using Mueller-Hinton Agar (MHA), which is the best medium for routine susceptibility tests because it has good reproducibility, low in sulfonamide, trimethoprim, and tetracycline inhibitors, and gives satisfactory growth of most bacterial pathogens.The inoculum for the disk diffusion method is prepared using a suitable broth such as tryptic soy broth. This medium is prepared according to manufacturer's instructions, dispensed in tubes at 4-5 ml and sterilized. Sterile 0.9% salt solution may also be used.
- Media are supplemented with 1-2% sodium chloride (NaCl) if intended for marine organisms
- All Test Samples of concentration 50µg/ml using DMSO as solvent against E. coli, Pseudomonas aeruginosa, Staphylococcus aureus & C. albicans with Nystatin & Gentamicin was used as a standard.

Table No.1: Data For Disc Diffusion Method Result

Compound code	E. coli Zone of inhibition(mm)	Pseudomonas aeruginosa Zone of inhibition(mm)	Staphylococcus aureus Zone of inhibition(mm)	C. albicans Zone of inhibition(mm)
QZAl	No Zone	No Zone	No Zone	No Zone
QZ4Cl	No Zone	No Zone	No Zone	22mm
QZ2Cl	No Zone	No Zone	No Zone	16mm
QZ3,4Cl	No Zone	No Zone	No Zone	20mm
QZ4Br	No Zone	No Zone	No Zone	15mm
QZ4Fl	No Zone	No Zone	13mm	12mm
QZ4Tl	No Zone	No Zone	No Zone	13mm
QZ2Tl	No Zone	No Zone	No Zone	18mm
QZ3Tl	No Zone	No Zone	No Zone	16mm
QZOTl	No Zone	No Zone	No Zone	11mm
Gentamicin	16mm	20mm	No Zone	-
Nystatin	-	-	-	18mm

- From the above observation the test compound shows zone of inhibition against Candida albicans
- Test compounds QZ4Cl and QZ3,4Cl show greater zone of inhibition compared to standard.
- Compound QZ42Tl showed same zone of inhibition as that of standard.Compounds QZ2Cl, QZ4Br, QZ4Fl, QZ4Tl , Q3Tl and QZOTl showed smaller zone of inhibition than that of standard.Compounds QZAl did not show any zone of inhibition that is fungal species is resistant to these compounds.

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