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

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ABSTRACT

Benzo-1,3-diazinebased 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 2,4-pentenedionein presence of catalyst $FeCl_3.6H_2O$(10mol%) results in 2-methyl4-(3H)-quinazolinone (I) which undergo chlorination reactionin presence of $SOCl_2$ converted to 4-chloro-2-methylbenzo-1,3-diazine (II).The Intermediate4-chloro-2-methylbenzo-1,3-diazine (II) condensed with different aromatic amines in presence of isopropyl alcohol (IPA) results in 4-amino substituted quinazoline derivatives. An efficient $FeCl_3.6H_2O$(10mol%) 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 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.

KEYWORDS: Benzo-1,3-diazine ,Quinazollinone, benzamide ,antimicrobial agents

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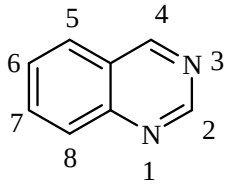
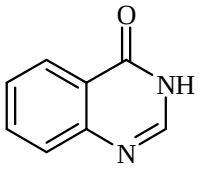
INTRODUCTION

Quinazoline is nitrogen containing fused heterocycles having considerable pharmaceutically interest because of their diverse range of biological properties. In heterocyclic rings number of groups can be substituted which can help for study of structure activity relationship and also for synthesize of new series of compounds.Quinazoline was first proposed by weddige.Chemically it is named as 1,3-diazenapthalene or 5,6-benzopyrimidine and its 4-oxo i.e. keto derivatives is known as 3(*4H*)-quinazolinone[1,2,3].



Quinazoline



4(3*H*)quinazolinone

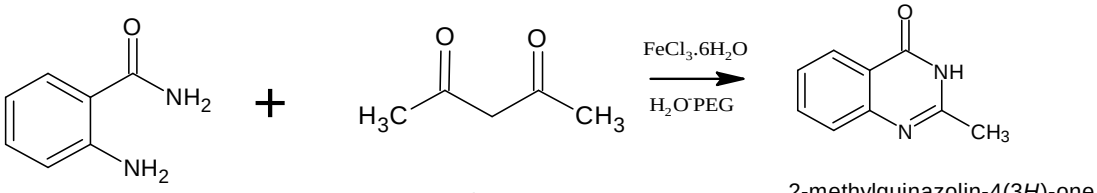
Quinazolinones have high melting crystalline solids , insoluble in water and organic solvent but soluble in aqueous alkali. Most appericiable thing of this ring system is its 'stability', as no degradation is shown by any simple chemical reactions such as oxidation ,reduction, hydrolysis reactions. Quinazoline-3(*4H*)-ones and derivatives have been known as promising class of biologically active compounds[4,5]. Due to stability of ring and versatile nature in pharmacological activities such as antibacterial[6,7], antitumour[8], antitubercular[9], anticonvulsant[10], much attention is paid on synthesis of potential medicinal agents by medicinal chemists.

ANTIMICROBIAL ACTIVITY

"Antimicrobial Activity may be defined as activity tending to destroy microbes, prevent their development or inhibit their pathogenic action.[11]Antimicrobial agents is defined as a compound that selectively destroys or inhibits the growth of microorganism.[12] 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.[13]Hosakera D. Revanasiddappa et al.' Synthesized a series of Quinazolinone compounds, having antimicrobial activity against the bacteria E.coli and S. aureus and fungi. Aspergillusniger and A. flavus.[14] 4-(3H)-quinazolinone which are most prevalent either as intermediate or as natural products in many proposed biosynthetic pathways.[15] This is partly due to the structure being derived from the anthranilates. 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.[16]

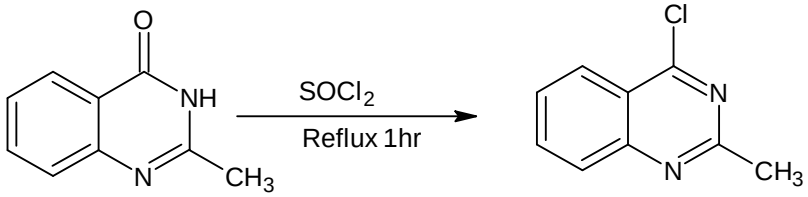
RESULTS AND DISCUSSION:

2-amino benzamide/anthranilamide (1) was treated with2,4-pentenedione in presence of anhydrous $FeCl_3.6H_2O$(10mol%) using sufficient quantity of solvent (PEG 400/H_2O 1:9) reflux for 12 hour to obtain 2-methyl-4(3H)-quinazolinone (I) it involve attack of nucleophile of electrophilic carbon of 2,4-pentenedione which lead to nucleophilic aromatic substitution type of reaction yields (I),a catalytic amount of $FeCl_3.6H_2O$(10mol%) is used i.e$FeCl_3.6H_2O$(10mol%) catalysed reaction it is avery clean reaction gives maximum yield of2-methyl-4-(3H)-quinazolinone(I)

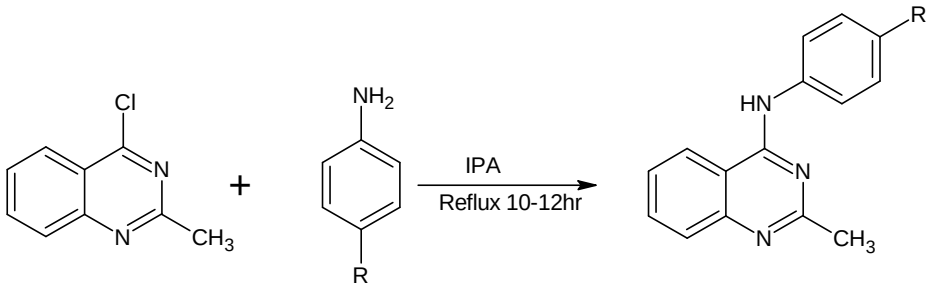


Anthranilamide Acetyl acetone 2-methylquinazolin-4(3*H*)-one

Intermediate (I) undergo chlorination reaction in presence of $SOCl_2$converted to 4-chloro-2-methyl-4-(3H)-quinazoline (II).2 methyl quinazoline-4(3H)-one was dissolve in dimethyl formaide (DMF), addition of distilled thionylchloride ($SOCl_2$) by drop wise at 0^0C with continuous stirring. After addition of $SOCl_2$reaction mixture refluxed under stirring for 1hr.Product was confirmed by TLC, melting point and chemical test.



The Intermediate II condensed with different aromatic amines in presence of Isopropyl alcohol (IPA) results in 4-amino substitutedbenzo-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.



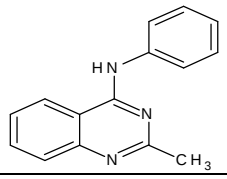
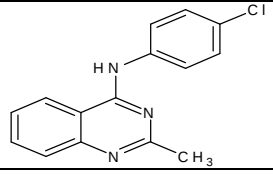
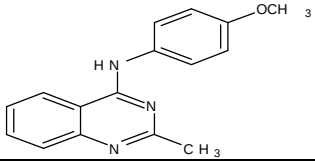
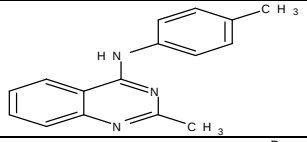
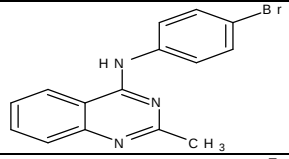
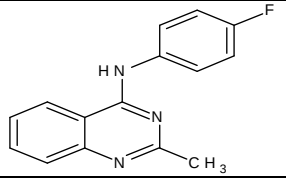
4-chloro-2-methylbenzo-1,3-diazine Substituted Aniline 4-substituted benzo-1,3-diazine

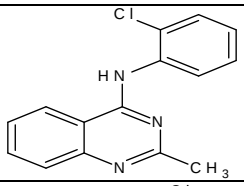
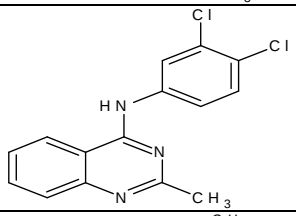
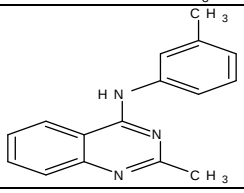
An efficient pyridine catalyzed approach ishas been used to developedquinazolinonederivatives, and the protocol uses cheap and readily available substituted 2-amino benzamide as the starting materials. This can be the example of constructing nitrogen containing six membered benzofusedheterocycles.

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	4-Pentenedione	$-CH_3$	Aniline	235	165-168	0.81	68.06
		$-CH_3$	4-Chloro Aniline	269	176-178	0.78	76.00
		$-CH_3$	4-Methylaniline	265	146-148	0.80	66.18
		$-CH_3$	4-Methoxyaniline	249	182-184	0.77	63.65
		$-CH_3$	4-Bromo Aniline	314	162-164	0.81	68.00
		$-CH_3$	4-Fluoro Aniline	253	168-170	0.79	73.00
		$-CH_3$	2-Chloro Aniline	269	137-139	0.78	71.00
		$-CH_3$	3,4-Chloro Aniline	304	145-148	0.82	65.00
		$-CH_3$	3-Methyl Aniline	249	137-139	0.78	69.00

Table 2. Structures For Synthesized Compounds

CODE	STRUCTURE	MOLECULAR FORMULA
Qzal*		$C_{15}H_{13}N_3$
QZ4Cl*		$C_{15}H_{12}N_3Cl$
QZ4A*		$C_{16}H_{15}N_3O$
QZ4Tl*		$C_{16}H_{15}N_3$
QZ4Br*		$C_{15}H_{12}N_3Br$
QZ4F*		$C_{15}H_{12}N_3F$

QZ2Cl*		$C_{15}H_{12}N_3Cl$
QZ34Cl*		$C_{15}H_{11}N_3Cl_2$
QZ3Tl*		$C_{16}H_{15}N_3$

EXPERIMENTAL METHOD:

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

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

Mix anthranilamide and 2,4-pentenedione, catalytic amount of $FeCl_3.6H_2O$(10mol%) using sufficient quantity of solvent (PEG 400/H_2O 1:9) reflux for 12hour.Cool the solution, filter it & wash with water to obtain 2-methyl-4(3H)-quinazolinone. Crude product recrystallized from ethanol (Yield: 86.00% M.P: 231-233^{0}C)

ATR 3170.97cm^{-1} (N-H stretch) ,3039.81cm^{-1} C-H stretch(aromatic), 1674.21 cm^{-1} C=O (amide) , 2908cm^{-1} (C-H stretch (CH_3)) , 1H NMR (500 MHz, $CDCl_3$) δ12.17(s, 1H) , δ6.490-8.219(8H),2.506 (s, 3H)) HRMS (m/z): [M + H]+, calcd for $C_9H_8N_2O$, 160 found, 160.1

4-chloro-2-methylbenzo-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 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-**methyl-4-(phenylamino)benzo-1,3-diazine(III):**

3- 4-Chloro-2-methylbenzo-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-methyl 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-methyl-4-(3,4-dichlorophenylamino)benzo-1,3-diazine (IV):

4-Chloro-2-methylbenzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.1M 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-methyl-4-(4-bromophenylamine)benzo-1,3-diazine (V):

3-4-Chloro-2-methylbenzo-1,3-diazine(II) 0.1M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.1M 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-methyl-4-[(4-fluorophenylamine)benzo-1,3-diazine (VI):

4-Chloro-2-methylbenzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.1M 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-**methyl-4-(4-methylphenylamine)benzo-1,3-diazine (VII):**

3-4-Chloro-2-methylbenzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.1M 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-methyl-4-(4-chlorophenylamine)benzo-1,3-diazine (VIII):

4-Chloro-2-methylbenzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.1 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-methyl-4-(2-chlorophenylamine)benzo-1,3-diazine (IX):

4-Chloro-2-methylbenzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.1 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-methyl-4-(4-methoxyphenylamine)benzo-1,3-diazine (X):

4-Chloro-2-methylbenzo-1,3-diazine(II) **0.1** M was dissolved in of iso-propyl alcohol (IPA). Drop wise 0.1 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 albicansATCC10231

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: 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
QZ4C*	No Zone	No Zone	No Zone	13
QZ4A*	No Zone	No Zone	No Zone	No Zone
QZ4Tl*	No Zone	No Zone	No Zone	18
QZ4Br*	No Zone	No Zone	No Zone	15
QZ4F*	No Zone	No Zone	No Zone	23
QZ2Cl*	No Zone	No Zone	No Zone	11
QZ3,4Cl*	No Zone	No Zone	No Zone	17
QZ3Tl*	No Zone	No Zone	No Zone	No Zone
Gentamicin	16mm	20mm	No Zone	-
Nystatin	-	-	-	18

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