SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY STUDIES OF (E)-N'-(2-(2-(SUBSTITUTED BENZALDEHYDE) HYDRAZINYL) QUINAZOLINE-4-YL) ISONICOTINOHYDRAZIDE

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Received October 01, 2013 *Accepted January 10, 2014*

ABSTRACT

The compounds have been synthesized by refluxing the *N*'-(2-hydrazinoquinazolin-4-yl)isonicotinohydrazide and different substituted aldehyde in presence of acid catalyst to yield (E)-N'-(2-(2-(substitutedbenzalidene) hydrazinyl) quinazolin-4-yl) isonicotino hydrazide using conventional methods and studied their biological activities.

Key Words : 2, 4-dichloroquinazoline, Isoniazid, IR/NMR spectroscopy, Antibacterial, Antifungal activities

INTRODUCTION

Schiff bases[1] are the important compound owing to their wide range of biological activities and industrial application. They have been found to posses the pharmacological activities such as antimalarial[2], anticancer[3], antibacterial[4], antifungal[5],antitubercular[6], anti-inflammatory, antimicrobial[7] and antiviral[8] etc. They also serve as a back bone for the synthesis of various heterocyclic compounds. The presence of azomethine and sulfonamide functional group is responsible for antimicrobial activities, which can be altered depending upon the type of substituent present on the aromatic rings. In view of these above biological importance of Schiff bases. We plan to synthesis of some novel sulphanamide analogs of Schiff bases by Schiff reaction.

The present work is oriented towards synthesis of some Schiff bases by condensing N'-(2-hydrazinoquinazolin-4-yl)isonicotinohydrazide with different aromatic aldehydes in the presence of Conc.H_2SO_4 and THF at 130-140^{0}C. All the synthesized compounds have been characterized on the basis of their m.p, TLC, IR and 1H NMR spectral data. The antimicrobial activities of these compounds was evaluated by broth dilution method.

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MATERIAL AND METHODS

All the chemicals used were of pure grade (Merck and B.D.H). The melting points of all complexes were determined by open capillary method and were uncorrected.

Experimental preparation of 2, 4-Quinazolinedione

In a 1000ml beaker, a mixture of (20 gm, 0.146mole) of anthranilic acid, 700ml of warm water (40^{0}C) and glacial acetic acid (11ml, 0.19mole) was stirred mechanically and allowed to cool to room temperature. A freshly prepared solution of (15gm, 0.185mole) of potassium cyanate in 50ml of water was then added drop wise with stirring over a period of 15 to 20 minutes. The resulting pasty mixture was stirred for 20 minutes and then (200gm, 5mole) of sodium hydroxide was added slowly in small portion. During this addition the reaction mixture was kept below 40^0 C by cooling in a cold water bath. A clear solution was obtained momentarily, but in a sort time a fine granular precipitate of the hydrated mono sodium salt of benzoylene urea

precipitate. After the mixture has cooled over night in an ice box, the precipitated sodium salt was collected on a Buckner funnel. The colorless salt was dissolved in 1 lit of hot water and the solution was filtered. The compound was precipitated by adding dilute sulfuric acid.(1:1) with vigorous stirring until the liquor was acidic to litmus the material was collected, washed with water and dried in an oven at 100 ^{0}C.

Preparation of 2, 4-Dichloroquinazoline

In a 250 ml R.B.F., a mixture of (20gm, 0.123mole) of 2, 4-quinazolinedione, 200ml of phosphorus oxychloride and of tri-n-propyl amine (59gm 0.226mole) refluxe for 30minutes to yield a clear solution and volatile liquid was removed by distillation. The remaining mass was added in to ice and isopropyl alcohol. The product was extracted from residue with (4x200 ml) portion of hot n-heptane containing 2% tri-n-propyl amine. The combine extracts, at room temperature was diluted with enough benzene to dissolved crystallized solid. The organic solution was washed with 400ml of 5% NaOH and three times with water. The solvent was removed in vacuo and the residual solid was recrystallized from 2:1 ethyl acetate: n-heptane to give 12.1 gm. Of 2,4-dichloroquinqzoline as white needles. A second crop of product was obtained by reducing the mother liquor to one fourth the volume to give 6.7 gm. The combine yield of the two crops was 18.8 gm.

Preparation of N'-(2-chloroquinazolin-4-yl) isonicotinohydrazide

In 250 ml R.B.F., a solution of 2,4-dichloroquinazolin(0.01 mole) dry ether(20 ml) was taken and added isoniazid (0.01 mole) the mixture was stirred at room temperature for 24 hr, the ether was removed on a steam bath and the residue dissolved in approximately 150 ml of water. The solution was made basic with 20 % aqueous NaOH and extracted three times with chloroform. The combined chloroform extract were washed once with water, once with saturated sodium chloride solution and then dried over magnesium sulphate. After removal of chloroform the residue was purified by recrystallization using ethanol.

Preparation of N'-(2-hydrazinoquinazolin-4-yl)isonicotinohydrazide

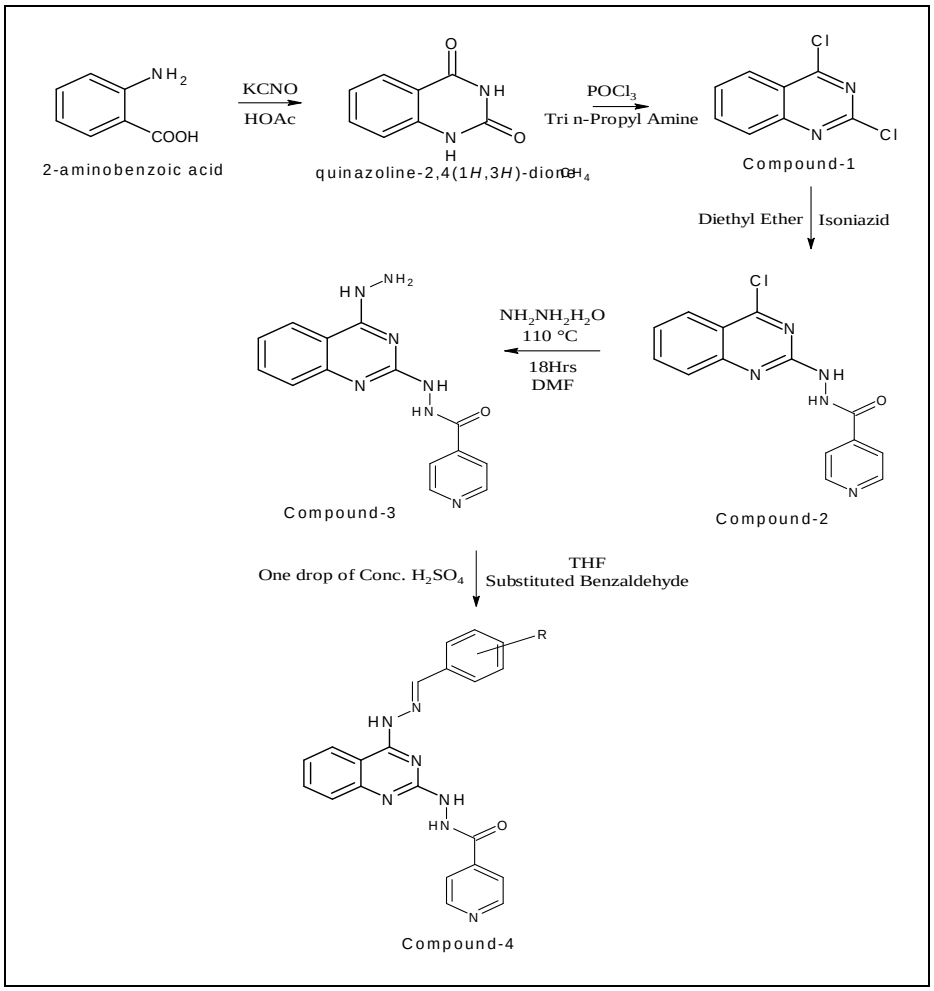
In a 250 ml R.B.F.,solution of N'-(2-chloroquinazolin-4-yl) isonicotinohydrazide. (0.01 mole) in DMF was taken and added Hydrazine hydrate (80%,0.01 mole).The reaction mixture was stirred at 110 ^{0} C for 18 hrs. The resulting mixture was cooled to room temperature, neutralized with ammonia. The solid was filtered, washed, dried and recrystallised using ethanol.

Preparation of E)-N'-(2-(2-(substituted benzalidene) hydrazinyl) quinazolin-4-yl) isonicotino hydrazide

In a 250 ml R.B.F., N'-(2-hydrazinoquinazolin-4-yl) isonicotinohydraz -ide (3.10 gm ,0.01 mole) in THF was taken and Substituted benzaldehyde (0.01 mole) and 1 drop of conc. H_2SO_4 were added and refluxed for 9 – 10 hrs. The completion of reaction was monitored by TLC examination 1:1 (Hexane: ethyl acetate). After completing of reaction the flask was cooled over night and residue was filtered off. The solid thus separated was filtered, washed with water and recrystalised from ethanol.

RESULTS AND DISCUSSION

E)-N'-(2-(2-(substitutedbenzalidene) hydraz-inyl) quinazolin-4-yl) isonicotino hydrazide were prepared in high yield in four steps from the reaction of anthranilic acid with glacial acetic acid and fresh potassium cynate which gives intermediate product which is further cyclised $POCl_3$ and tri-N-propyl amine to yield compound-1.This compound-1 on treatment with isoneazid and diethyl ether to yield compound-2. Compound-3 was prepared by reaction of hydrazine hydrate and compound-2 where compound-4 is prepared by coupling of compound -3 and substituted benzaldehyde the compound-4 was easily obtained and characterized by spectral analysis**(Fig. 1)**. The compound -4 was confirmed from their 1H-NMR spectral. **(Table 1)**



Where R = 4-CH_3,3-OCH_3-4-OH, 4-Cl, 2-NO_2, 2:4-Cl_2, 2-OCH_3, 4-NO_2, 4-H, 4-N $(CH_3)_2$, 2-OH

Fig. 1 : Reaction scheme

Table 1 : Characterization table of E)-N'-(2-(2-(substituted benzalidene) hydrazinyl) quinazolin-4-yl) isonicotino hydrazide

No	R	Molecular formula (M. wt.)	Yield (%) (per./ hrs.)	M.P. ^{0}C
4a	4-CH_3	$C_{24}H_{21}N_5O$ (395.47)	78 (9)	157-58
4b	3-0CH_3,4-OH	$C_{24}H_{21}N_5O_3$ (427.46)	72 (9)	160-61
4c	2-NO_2	$C_{23}H_{18}N_6O_3$ (426.44)	82 (10)	205-06
4d	4-Cl	$C_{23}H_{18}ClN_5O$ (415.88)	68 (9.5)	174-75
4e	2,4-$(Cl)_2$	$C_{23}H_{17}Cl_2N_5O$ (450.32)	75 (10)	152-53
4f	2-OCH_3	$C_{24}H_{21}N_5O_2$ (411.46)	65 (9.5)	188-89

4g	4-NO_2	$C_{23}H_{18}N_6O_3$ (426.44)	86 (10)	203-04
4h	4-H	$C_{23}H_{19}N_5O$ (381.44)	70 (9)	145-46
4i	4-$N(CH_3)_2$	$C_{25}H_{24}N_6O$ (424.51)	59 (9)	198-9
4j	2-OH	$C_{23}H_{19}N_5O_2$ (397.44)	84 (10)	163-64

^{1}H-NMR spectra

The NMR spectra of the compound-4a gave the multiplate between 5.91-7.80 δ ppm for aromatic protons, singlet at 2.5035 δ ppm for methyl group (-CH_3-) , singlet at 3.6499 δ ppm for –alkyl amine(-NH-) of isoniazd , singlet at 8.1507 δ ppm for –alkyl amine(-NH-) of hydrazine hydrate singlet at 9.6540 δ ppm for –alkyl amine(-NH-) of hydrazine hydrate.Thus the structure of compound is confirmed.

Infrared spectra

The IR Spectra of the compound-4a gave N-H (st) at 3292.96, –CH_3 (substitution-methyl group) at 2884.14,-C=O (isoneazid) at 1736.65, aromatic C=C at 1597.80, -C=N (st) quinazoline at 1611.30, -N-C-N (st) quinazoline at 1362.51,in plane Ar-H 1107.95,Ar-h (b) Vib. At 844.70, out plane Ar-H at 693.31 where the IR spectra of the compound-4c gave N-H (st) at 3226.46, –NO_2 (substitution-nitro group) at 1309.48, -C=O (isoneazid) at 1734.76, aromatic C=C at 1581.40, -C=N (st) Quinazoline at 1618.05, -N-C-N (st) Quinazoline at 1364.44,in plane Ar-H 1088.66,Ar-h (b) Vib. At 847.59, out plane Ar-H at 704.88

Antimicrobial activities

Antimicrobial and antifungal activities of compound 4a to 4j was investigated via the broth dilution method.[9-11] Bacteria strains *S.aureus, E. coli, P.aeruginosa* and *S. pyogenus* were used. Microorganisms were cultured on water aminopeptide solution (pH 7.2).The amount of bacteria in 1 ml of solvent was 2.5105 colony forming unit (c.f.u.) after 18 h of treatment. Antimicrobial activities were estimated by Minimum Inhibitory Concentration (MIC) the lowest concentration to completely inhibit bacterial growth of the compound shown in mg/ml. Compounds with MIC500mg/ml were considered to be in-active. Every experiment was repeated three times **(Table 2)**.

Table 2 : Antibacterial activities of compound

Code No.	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**Ampicillin**	**Chloramphenicol**
S. aureus	50	250	100	25	50	100	250	100	200	62.5	250	50
P.aeruginosa	500	500	250	200	100	100	50	250	500	100	100	50
E. coli	100	62.5	125	100	500	500	250	250	200	250	100	50
S. pyogenus	100	200	200	62.5	250	500	500	250	100	200	100	50

The investigation of antifungal activity of Compound 4a to 4j was carried out with the stiff plate agar diffusion method against *C.albicans,A.niger* and *A.clavatus.* The amount of microbial cells was 109 c.f.u./ml. Incubation period was 24 h at 35 °C for bacteria. Antibiotics nystatin, greseofulvin were used as standards. The bacterial cultures, standards and obtained substances in 5 mg/ml concentration were streaked across grooves and then allowed to dif-fuse in the agar nutrient plate **(Table 3)**.

Table 3 : Antifungal activities of compound

Code No.	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	Nystatin	Greseofulvin
C.albicans	500	100	1000	250	1000	1000	1000	500	100	250	100	500
A.niger	1000	50	1000	500	500	500	1000	100	100	50	100	100
A.clavatus	1000	50	1000	500	1000	100	1000	100	100	50	100	100

The antimicrobial effect and degree of activity of the tested compounds were evaluated by measuring the zone diameters and the results were compared with well known drugs **(Table 1)**. Every ex-periment was repeated three times.

CONCLUSION

The work has approached towards the synthetic and biological approach of these wonder molecules. Anti-bacterial properties of the synthesized compounds has exhibited very good inhibition, all compounds have exhibited well to moderate activity towards *E. coli* except compound 4e and 4f as compared with standard ampicillin.Compound 4a, 4d and 4e is active against *S.aureus,* as compare to standard chloremphenicol and compound 4b, 4g and 4i is in-active against *S.aureus,* as compare to standard Ampicillin. Compound 4e, 4f and 4j is active against *P.aeruginosa* as compared to standard Ampicillin. compound 4a, 4d and 4e is active against *S. pyogenus* as compare to standard ampicillin. But the systematic substitution at various position and other derived compounds have shown remarkable antifungal properties. The compounds 4b, 4i and 4j have exhibited outstanding activity towards *C.albicans, A.niger* and *A.clavatus*. The remaining compounds have shown poor antifungal activity indicating less biological importance for a synthetic chemist. Efforts are under progress in evaluation of these synthesized compounds for *in vivo* studies especially the anti tubercular and anti-malarial agents and the results will be published in later communications. This class of compounds has a great scope compared to other organic moieties because of their mesoionic nature, solubility and high sensitiveness towards the biological behaviors.

ACKNOWLEDGEMENT

The authors are thankful to Department of Chemistry, Arts, Science and Commerce College, Kholwad, Surat, India for providing laboratory facilities.

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