BRIDGEHEAD NITROGEN HETEROCYCLIC SYSTEMS: FACILE SYNTHESIS, BIOACTIVITY OF SOME NEWER DERIVATIVES OF 1-SUBSTITUTED BENZYLIDENE HYDRAZINO TETRAZOLO [1, 5-*a*] QUINOXALINES.

Umarani N* and Ilango K
Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM University, Kattankulathur - 603 203, Kancheepuram District, Tamil Nadu, India.
***E-mail:** umaarun79@gmail.com

ABSTRACT:

The present work addresses a search for new lead antimicrobial agents by adopting a simple procedure for the synthesis of new series of Tetrazolo quinoxalines. o-Phenylene diamine was condensed with oxalic acid using Phillip's procedure to obtain quinoxaline-2, 3-dione. The former on chlorination followed by hydrazinolysis furnishing the formation of hydrazides, which undergoes cyclisation with sodium azide afforded 2-hydrazino tetrazolo [1,5-*a*] quinoxaline. The scaffold synthesized was made to react with different aromatic aldehydes resulted in the formation of 1-substituted benzylidene hydrazino tetrazolo-[1,5-*a*]-quinoxalines. Their chemical structures have been confirmed by IR, ^{1}HNMR and MS spectral studies and elemental analysis. Investigation of *in vitro* antimicrobial activity of compound was done by cup-plate agar diffusion method. Some of the obtained compounds showed the interesting antimicrobial activity comparable to standard drugs Ofloxacin and Griseofulvin. The ambient conditions, excellent product yields and easy work up procedures make this synthetic strategy a better protocol for the synthesis of newer schiff's derivatives.

Keywords: Tetrazolo quinoxalines; Scaffold; Spectral studies; Synthetic strategy.

INTRODUCTION

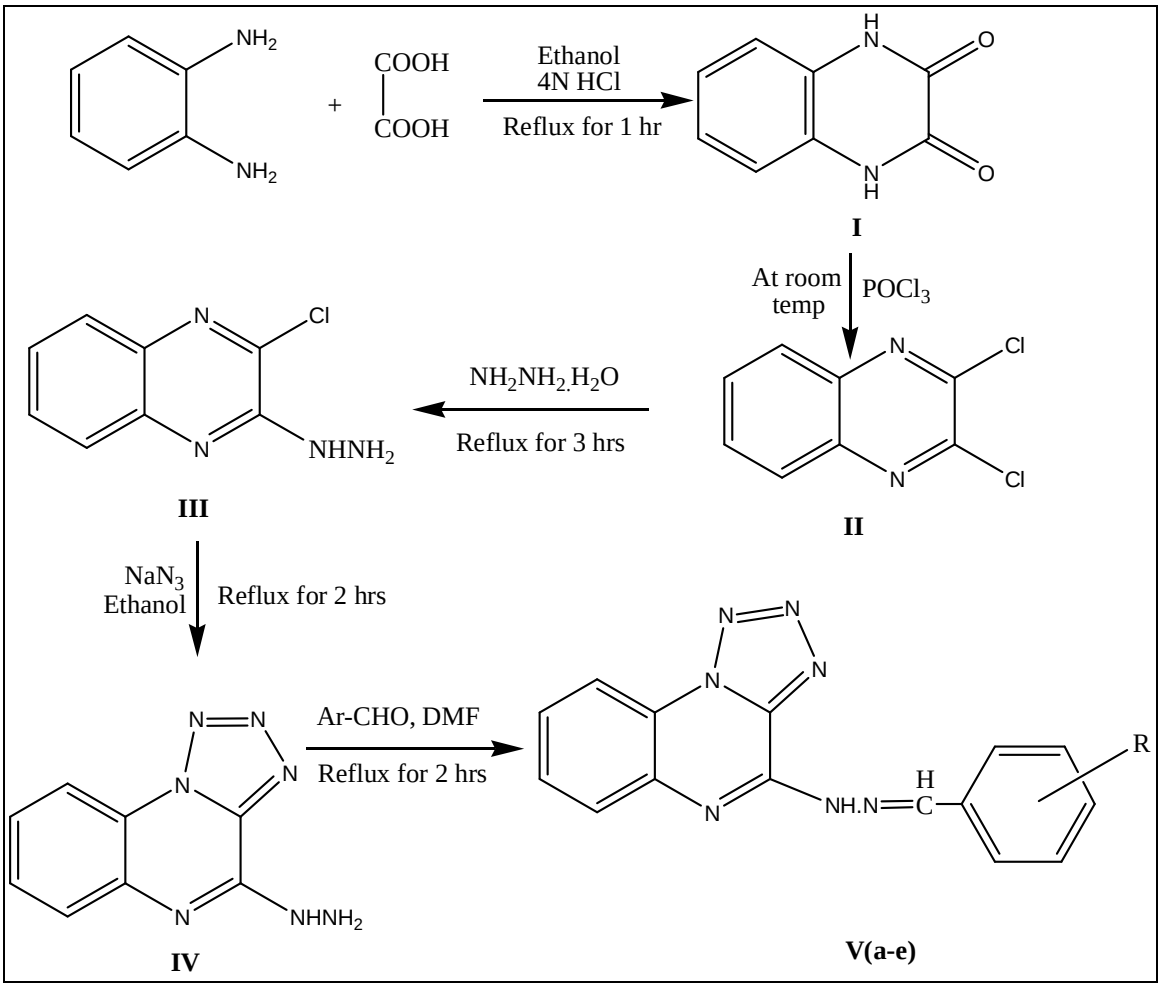
In recent years tetrazoles fused with quinoxaline ring have been grabbing the attention of the synthetic chemists for their multifaceted pharmacological and medicinal applications. A perusal of literature has revealed that quinoxalines, in particular display wide gamut of biological activities such as antiviral[1] (Hepatitis B), antimicrobial[2], antidepressant[3], tuberculostatic[4], antidiabetic[5], anti-inflammatory[6], analgesic[7], antiallergy[8], anticancer[9], diuretic[10], anticonvulsant[11] and muscle relaxant[12] activities. Moreover fusion of tetrazole, which is considered as planar acidic heterocyclic analogue of carboxylic function[13, 14] has the ability to increase potency[15, 16] and improve bioavailability[17]. In the light of above facts and expecting an enhanced bioactivity from coupling of two heterocycles, we herein report the synthesis of newer bridgehead nitrogen heterocyclic systems bearing tetrazolo quinoxalines and their antimicrobial evaluation against pathogenic microorganisms at different concentrations using the cup-plate agar diffusion method.

Chemistry

The target compounds were synthesized according to the representative Scheme-1. The required starting material, quinoxaline-2,3-dione (I) was prepared in good yield by condensation of o-Phenylene diamine with oxalic acid in 4N HCl using Phillip's procedure[18]. The subsequent chlorination reaction of (I) with phosphorous oxychloride at room temperature gave the known 2,3-dichloro quinoxaline (II), which on hydrazinolysis afforded the corresponding 3-chloro-2-hydrazino quinoxaline (III). The compound (III) undergoes cyclisation with sodium azide in ethanol to obtain 2-hydrazino tetrazolo [1,5-*a*] quinoxaline (IV). The scaffold synthesized was finally treated with different substituted aromatic aldehydes resulted in the formation of schiff's bases of title compounds V(a-e). The yields of all the synthesized compounds were found to be in the range of 78-86%. Thin layer chromatography was run through out the reaction to optimize the reaction completion. The physical data of the titled compounds V(a-e) were recorded in Table 1.

Table- 1: Physical data of titled compounds V(a-e).

Compound code	Ar	Molecular Formula	Molecular Weight	R_f Value	Melting Point (^oC)	Percentage Yield (%)
Va	-C_6H_5OH (2-Hydroxy phenyl)	$C_{15}H_{11}N_7O$	305.31	0.69	254-257	83
Vb	-$C_6H_5NO_2$ (4-Nitro phenyl)	$C_{15}H_{10}N_5O_2$	334.11	0.66	296-298	80
Vc	-C_6H_5Cl (3-Chloro phenyl)	$C_{15}H_{10}O_7Cl$	323.76	0.72	305-307	78
Vd	-$N(CH_3)_2C_6H_5$ (2-Dimethyl amino phenyl)	$C_{17}H_{16}N_5$	332.15	0.63	284-286	82
Ve	- $(OCH_3)_3C_6H_3$ (3,4,5-Trimethoxy phenyl)	$C_{18}H_{17}N_7O_3$	379.14	0.77	298-302	86



Scheme-1: Synthesis of 1-substituted benzylidene hydrazino-2-tetrazolo [1,5-*a*] quinoxalin–4-yl) derivatives V(a-e)

IR Spectral Studies

Assignments of selected characteristic IR band positions provide significant indication for the formation of 1-substituted benzylidene hydrazino tetrazolo [1,5-*a*] quinoxalines. The infrared spectroscopic investigation of all the compounds V(a-e) showed sharp peak at 3340-3420 cm^{-1} due to the presence of NH stretching of NH_2, an intense band of 1618 cm^{-1} to 1632 cm^{-1} attributed to the appearance of -N=N stretching, sharp bands at 1523cm^{-1} and 1561cm^{-1} evidenced the appearance of -C-N and -C=N stretching. The asymmetric vibrations of the aromatic nitro group in the compound Vb exhibited a peak of medium intensity at 1565cm^{-1}.

NMR Spectral Studies

Further evidence for the formation of target compounds was obtained from the ^{1}HNMR spectra, which proved to be a diagnostic tool for the positional elucidation of the proton. Assignments of the signals are based on the chemical shift and intensity pattern. The formation of schiff's bases was evidenced by the disappearance of NH_2 protons and appearance of azomethine (-N=CH) proton peak at δ ppm 8.42. The multiplet signals at δ ppm 7.23-8.45 are the characteristics of aromatic ring protons. A sharp signal appears at δ ppm 3.92 is the characteristics of the protons of -OCH_3. A broad signal at δ ppm 11.42, due to the characteristics of -NH proton was accordance with all the proposed structures.

Mass spectral analysis

Final proof for the structures were obtained by recording its mass spectrum, which exhibited a molecular ion peak at m/z 305, 334, 323, 332, and 379 respectively corresponding to its molecular weight. The mass spectral data of all the titled compounds were found to be in correlation with the expected structure.

Antimicrobial Activity

The antimicrobial activity was assayed by using cup-plate diffusion method[19] by measuring the inhibition zones in mm. Compounds **V(a-e)** were screened *in vitro* for their antimicrobial activity against a variety of bacterial strains such as *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* and fungal strains such as *Aspergillus niger* and *Candida albicans* at different concentrations of 100, 200, 300mg/ml. Known antibiotics like Ofloxacin and Griseofulvin were used as standard for comparison.

Antibacterial Activity

The purified title products were screened for its antibacterial activity. The nutrient agar broth prepared by the usual method[20] was inoculated especially with 0.5ml for 24 hours old subculture of all the mentioned bacterial

strains were taken in a separate conical flask at 40-50^{o}C and mixed well by gentle shaking. About 25ml of the contents of the flasks were poured and evenly spread in a petridish (13cm in diameter) and allowed to set for 2 hours. The cups (10mm in diameter) were formed by the help of borer in agar medium and filled with synthesized samples dissolved in dimethyl formamide(DMF). The plates were incubated at 37^{o}C for 24 hours and control was also maintained in similar manner. The zone of inhibition of the bacterial growth are measured in mm diameter and are recorded in Table 2.

Antifungal Activity

Aspergillus niger and *Candida albicans* were employed for testing fungicidal activity using cup plate method[21]. The cultures were maintained on Sabouraud's agar slants. Sterilized Sabouraud's agar medium was inoculated with 72 hours old suspension of fungal spores in a separate flask. About 25ml of the inoculated medium was evenly spread in a sterilized petridish and allowed to settle down for 2 hours. The cups (10mm in diameter) were punched in petridish and loaded with sample solution in DMF. The plates were incubated at room temperature (30^{o}C) for 48 hours. After the completion of the incubation period, the zone of inhibition of growth of compounds **V(a-e)** in the form of diameter in mm were measured. Along the test solution in each petridish, one cup was filled with solvent which acted as control. The antifungal activity of compound was compared with known standard drugs mentioned above, which are recorded in Table 2.

Table 2. In vitro Antimicrobial profile of the titled compounds (Va-Ve).

Compounds	Concentration (mg/ml)	Antibacterial activity Zone of Inhibition (mm)				Antifungal activity Zone of Inhibition (mm)	
		S.aureus	K.Pneumoniae	E.coli	P.aeruginosa	A.niger	C.albicans
Va	100	13	13	13	13	24	25
	200	15	14	15	14	27	28
	300	18	17	17	16	29	31
Vb	100	16	17	15	16	24	26
	200	18	20	19	19	27	28
	300	20	22	21	23	28	30
Vc	100	18	17	16	17	24	27
	200	20	19	18	23	26	29
	300	23	22	21	25	29	30
Vd	100	13	13	15	14	15	15
	200	17	15	17	16	16	17
	300	18	17	19	18	18	19
Ve	100	15	16	13	13	18	18
	200	16	18	15	15	24	20
	300	17	20	18	17	25	22
Ofloxacin	100	40	37	36	35	-	-
	200	44	39	38	37	-	-
	300	46	41	40	38	-	-
Griseofulvin	100	-	-	-	-	34	37
	200	-	-	-	-	36	39
	300	-	-	-	-	37	40

The results of in vitro antimicrobial activities were summarized in Table 2. It was observed that the synthesized compounds having electron withdrawing groups showed very good antimicrobial properties. The target compound **Vb** was excellently equipotent against the microbial strains, **Vc** showed optimum equipotent activity, **Vd** was moderately active and **Ve** was mild active against the strains. Thus the substitution of electron withdrawing groups in C-2 and C-4 of the phenyl ring seems to be of great significance for antimicrobial efficacy.

In conclusion, a new class of tetrazolo quinoxalines heterocycles was synthesized and the results of antimicrobial data revealed that the compounds possess significant *in vitro* activity. Therefore, this study would be a fruitful matrix for the development of novel class of schiff's bases of tetrazolo quinoxalines as interesting lead molecules for further synthetic and biological evaluation. It is convincing that this class of compounds certainly

holds great promise towards the pursuit to discover novel class of antimicrobial agents. Further studies to acquire more information concerning structural activity relationships are in progress.

EXPERIMENTAL

All chemicals used were of analytical grade and purchased from SD Fine. Chemicals (Mumbai, India). The melting point of the compounds was determined on a Veego digital melting point apparatus (Model VMP-D) and the values are uncorrected. The purity of the compounds and progress of reaction was monitored by thin layer chromatography (TLC) using precoated silica gel G aluminium plates (0.5mm thickness, E-Merck) and spots were visualized using UV radiation/iodine vapour. Infra red absorption spectra were scanned in the 4000-400cm^{-1} ranges using KBr discs on FTIR Shimadzu Spectrometer. ^{1}HNMR spectra were scanned at 300 MHZ on a Bruker Avance II Model Spectrophotometer using TMS as internal reference standard and chemical shifts (δ) are quoted in ppm. Mass spectra were acquired on Shimadzu GC-MS QP-2010. The elemental analysis of the titled compounds was carried out on a Vario EL analyzer.

GENERAL METHODS

Synthesis of Quinoxaline-2, 3 dione (I).

o-Phenylene diamine (27.9g, 0.25mol), oxalic acid (32.5g, 0.36 mol) and 4N HCl (150ml) were refluxed in an oil bath for 1 hr and cooled. The solid separated was filtered, washed and recrystallised with rectified spirit. Yield (86%), mp. >300^{o}C, MS: (M^{+}) 162.

Synthesis of 2, 3-dichloro quinoxaline (II).

Equimolar quantity of quinoxaline-2, 3-dione **(**16.013g, 0.1 mol), on treatment with phosphorous oxytrichloride (15.333g, 0.1mol) at room temperature afforded 2, 3-dichloro quinoxaline. The product obtained was recrystallised from rectified spirit. Yield 82%, mp: 264-268^{o}C, (M^{+}) 197.

Synthesis of 3-chloro -2 hydrazino quinoxaline (III).

To 2, 3-dichloroquinoxaline (2.98g, 0.015mol) and hydrazine hydrate (1g, 0.02mol) in 50 ml of ethanol were refluxed for 3 hours. The product obtained was filtered and recrystallised from rectified spirit. Yield (84%), mp. 187-190^{o}C, Mass (m/z): 194.

Synthesis of 2-hydrazino tetrazolo-[1, 5-a]-quinoxaline (IV).

To 3-chloro-2-hydrazino quinoxaline (3.89gm, 0.02mol) and sodium azide (1.30 gm, 0.02mol) were refluxed for 2 hours in an oil bath and with routine labor work-up yielded 2-hydrazino tetrazolo-[1,5-a]- quinoxaline. Yield 83%, mp: 187-189^{o}C, Mass (m/z): 199.

Synthesis of 1-substituted benzylidene hydrazino-2 (tetrazolo- [1, 5-a] quinoxalin-4-yl derivatives V(a-e).

The compound **IV** (2.01 gm, 0.01mol) and various aromatic aldehydes (0.010mol) on refluxing with DMF afforded the solid products in good yields, recrystallised with methanol and dried.

Va: 1- [(2-hydroxy benzylidene) hydrazino)-2-tetrazolo [1, 5-*a*] quinoxaline)] IR (KBr) cm^{-1}: 3020 (Ar-CH), 3344 (NH), 1618 (N=N), 1561 (C=N), 1523 (C-N), 3525 (-OH); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.32-8.40 (m, 8H, Ar-H), 11.82 (s, 1H, NH), 8.24 (s, 1H, CH), 8.43 (s, 1H, -OH); Mass (m/z): 305. Anal Calcd for $C_{15}H_{11}N_7O$ % C, 59.80; H, 4.71; N, 30.51; O, 4.98, Found: C, 59.84; H, 4.74; N, 30.53; O, 4.96.

Vb: 1-[(4-nitro benzylidene) hydrazino)-2-tetrazolo [1, 5-*a*] quinoxaline)] IR (KBr) cm^{-1}: 3028 (Ar-CH), 3420 (NH), 1632 (N=N), 1622 (C=N), 1534 (C-N), 1565 (Ar-NO_2); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.62-7.80 (m, 8H, Ar-H), 11.44 (s, 1H, NH), 8.78 (s, 1H, CH), Mass (m/z): 334. Anal Calcd for $C_{15}H_{10}N_8O_2$ % C, 54.85; H, 4.03; N, 31.98; O 9.13, Found: C, 54.86; H, 4.05; N, 31.94; O, 9.17.

Vc: 1- [(4-chloro benzylidene) hydrazino)-2-tetrazolo [1, 5-*a*] quinoxaline)] IR (KBr) cm^{-1}: 3052 (Ar-CH), 3343 (NH), 1628 (N=N), 1652 (C=N), 1156 (C-N), 796 (C-Cl); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.34-8.42 (m, 8H, Ar-H), 11.84 (s, 1H, NH), 8.92 (s, 1H, CH), Mass (m/z): 323. Anal calcd for $C_{15}H_{10}O_7Cl$ % C, 56.56; H, 4.15; Cl, 10.43; N, 28.86, Found: C, 56.54; H, 4.12; Cl, 10.40; N, 28.88.

Vd: 1-[(2-dimethyl amino benzylidene) hydrazino)-2-tetrazolo [1,5-*a*] quinoxaline)] IR (KBr) cm^{-1}: 3056 (Ar-CH), 3348 (NH), 1628 (N=N), 1548 (C=N), 1174 (C-N), ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.23 -8.42 (m, 8H, Ar-H), 12.14 (s, 1H, NH), 8.42 (s, 1H, CH), 3.84 (s, 6H, N$(CH_3)_2$); Mass (m/z): 332. Anal Calcd for $C_{17}H_{16}N_8$ % C, 62.05, H, 5.79; N, 32.16, Found: C, 62.08; H, 5.84; N, 32.18.

Ve: 1-[(3,4,5-trimethoxy benzylidene) hydrazino)-2-tetrazolo [1, 5-*a*] quinoxaline)] IR (KBr) cm^{-1}: 3042 (Ar-CH), 3298 (-NH), 1592 (N=N), 1562 (C=N), 1538 (C-N), 1040 (-C-OCH_3); ^{1}HNMR ($CDCl_3$, DMSO-d_6): δ 7.46 - 8.62 (m, 6H, Ar-H), 12.32 (s, 1H, NH), 8.62 (s, 1H, -CH), 3.8 (s, 9H, $(OCH_3)_3$); Mass (m/z): 379. Anal Calcd for $C_{18}H_{17}N_7O_3$ % C, 57.71; H, 5.35; N, 24.80; O, 12.14, Found: C, 57.74; H, 5.38; N, 24.83; O, 12.18.

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