SYNTHESIS OF SOME PHENYLPYRAZOLO BENZIMIDAZOLO QUINOXALINE DERIVATIVES AS POTENT ANTIMICROBIAL AGENTS

CH.SRIDEVI*, K.BALAJI[1], A.NAIDU[2] AND R.SUTHAKARAN[3]

*,1 Dept. of Pharmaceutical Chemistry, Geethanjali College of pharmacy, Cheeryal(v), Keesara (M), R.R (Dt).-501301

[2]Dept. of Pharmaceutical Chemistry, JNTUH, Kukatpally, Hyderabad.

[3]Dept. of Pharmaceutical Chemistry, S.R.R.College of pharmacy, Mahaboobnagar.

ABSTRACT

2,3-Diphenyl quinoxaline (NI) is fused with benzimidazole (NII) by a methylene bridge, which is then allowed for acetylation. The acetylated product (NIV) is made to react with different aromatic aldehydes to give chalcones (NV 1-NV 5). Chalcones refluxed with substituted acid hydrazides to afford different phenyl pyrazolo benzimidazole quinoxaline derivatives (NVI 1-NVI 15). The structure of chalcones and phenyl pyrazolo benzimidazole quinoxaline derivatives were confirmed by M.P, TLC and Spectral data. All the synthesized compounds were screened for their antibacterial and antifungal activities and results were presented.

KEY WORDS : 2,3-Diphenyl quinoxaline, benzimidazole, phenyl pyrazolo benzimidazolo quinoxaline, antibacterial activity, antifungal activity.

1.INTRODUCTION

Benzimidazole moiety plays an important role in heterocyclic chemistry largely due to its wide range of biological activities (Joshi,1989; Marijana,2008; Mader,2008;George,1997) such as antimicrobial, antitubercular, anti-inflammatory, anticancer, antihistamic etc., Quinoxaline derivatives have been reported to possess a wide variety of biological activities(Ganapathy,2007;George,2008) . Notable among these are antioxidant, anti-inflammatory antimicrobial, anticancer and antihistamic activities. Drugs having pyrazoline ring system (Asuncion,2007; Ragabasaw,2007;Harinadga babu,2004)araj are well known for their anti-inflammatory, antioxidant, antihistamic, antimicrobial, antidepressant, hypoglycemic, hypotensive, anticarcinogenic activities etc. In view of the above facts, it was contemplated to design and synthesize some phenyl pyrazolo benzimidazolo quinoxaline derivatives by condensing benzimidazole quinoxaline chalcones with different aromatic acid hydrazides (Scheme-I). All the synthesized compounds were screened for their antibacterial and antifungal activities. The structure of chalcones and phenyl pyrazolo benzimidazolo quinoxaline derivatives were confirmed by M.P, TLC and Spectral data.

***Corresponding author**
Assistant professor,
Flat no: 101, Vijay vihar appartments,
Opp. Mahatma Gandhi Law College,
N.T.R Nagar,L.B.nagar,
Hyderabad, Andhra Pradesh
Mobile: 09866870364
Email:sridevi.phd@gmail.com

2.MATERIALS AND METHODS

The melting point of the compounds were determined on a Thoshniwal electric melting point apparatus and the values were uncorrected. I.R spectrs of the compounds were recorded on a Thermo Nicolet Nexus670-FTIR, IICT, Hyderabad using KBr disc method. 1H NMR spectra were recorded onAvance-300, IICT, Hyderabad using $CDCl_3$ as solvent. Mass spectra were recorded on HITACHI RMU GL, IICT, Hyderabad. All the solvents used were of analytical grade.

EXPERIMENTAL SECTION

General procedure for synthesis of 6-((1H-benzo[d]imidazol-5-yl)methyl)-2,3-diphenylquinoxaline, NIII

2,3 Diphenyl Quinoxalin (NI) and benzimidazole (NII) were prepared following the literature method. NI and NII are linked with a methelyne bridge by treating equimolar quantities of NI and NII in suitable solvent with 35 parts formaldehyde solution and 35% HCl, stirring for 4 hr. at 70°C using magnetic stirrer. Solution was made alkaline using ammonia solution. Filtered the product and recrystallized with aq.ethanol.

NIII :Yield: 72%, m.p: 108°C, IR (KBr) in cm^{-1}: 1665 (C=N str.), 1340 (C-N str.), 3085 (Ar-H str.) .^{1}HNMR ($CDCl_3$) δ: 5.0 (S, 1H, N-H of benzimidazole), 3.81

(S, 2H, methylene), 7.5-7.9 (m, 3H, quinoxaline), 7.2-7.4 (m, 10H, Ar-H), 7.0-8.1 (m, 3H, benzimidazole). Mass: m/z: 428 (M+).

General procedure for synthesis of 1-(5-((2,3-diphenylquinoxalin-6-yl)methyl)-1H-benzo[d]imidazol-1-yl)propan-2-one, NIV

A solution of NIII (0.01M) and chloroacetone (0.01M) were taken into 250ml round bottom flask. Added to it 150ml of dry acetone and 30g of anhyd. Potassium carbonate and the reaction mixture were refluxed for 6hr. below 75°C. Filterate obtained was concentrated under vaccum and recrystallized with aq.ethanol(Leonard,2005).

NIV :Yield: 68%, m.p: 125°C, IR (KBr) cm^{-1}: 1793 (C=O str.), 1668 (C=N str.), 1340 (C-N str.), 3085 (Ar-H str.), 3323 (C-H str).^{1}HNMR ($CDCl_3$) δ: 2.0 (S, 3H, methyl), 3.8,4.8 (S, 4H, methylene), 7.4-7.9 (m, 3H, quinoxaline), 7.2-7.4 (m, 10H,Ar-H), 7.0-8.0 (m, 4H, benzimidazole). Mass: m/z: 468.2 (M+).

General procedure for synthesis of (Z)-4-phenyl-1-(5-((2,3-diphenylquinoxalin-6-yl)methyl)-1H-benzo[d]imidazol-1-yl)but-3-en-2-one, NV1-NV5

Method of aldol condensation followed. A solution of NaOH / KOH (8ml, 10% in water) was added drop wise to a well-stirred solution of N IV (0.01M) and (0.01M) of appropriate aldehyde in 20ml ethanol. The reaction mixture was stirred for 24hr. at cold conditions. Then diluted with ice water and acidified with Con.HCl. Filtered the product and recrystallized with aq.ethanol(Suthakaran,2007). The purity of the compound was checked by TLC and melting point.

NV 1: Yield: 73%, m.p: 113°C, IR (KBr) cm^{-1}: 1773 (C=O str.), 1668(C=N str.), 1340 (C-N str.), 3085 (Ar-H str.), 3323 (C-H str.) cm^{-1}.^{1}HNMR ($CDCl_3$) δ: 3.8, 5.3 (S, 4H, methylene), 6.2,7.3(d, 2H,ethylene), 7.5-7.9(m, 3H, quinoxaline), 7.1-7.4 (m, 15H, Ar-H), 7.0-8.1 (m, 4H, benzimidazole). Mass: m/z: 556.2 (M+).

General procedure for synthesis of 6-((1-((1-benzyl-4, 5-dihydro-5-phenyl-1H-pyrazol-3-yl) methyl)-1H Benzo [d]imidazol-5-yl) methyl)-2, 3-diphenylquinoxaline, NVI1– NVI15

Chalcone (0.01M) and aromatic acid hydrazide (0.02M) were taken in 20ml glacial acetic acid and refluxed for 10hr. above 130°c. The reaction mixture was concentrated and poured in 300ml of ice-cold water and recrystallized with aq.ethanol(Harinadha babu,2007). The purity of the compound was checked by TLC and melting point.

NVI 1: Yield: 67%, m.p: 121°C, IR (KBr) cm^{-1}: 1790 (C=O str.), 1668 (C=N str.), 1339 (C-N str.), 3035 (Ar-H str.), 3320 (C-H str.) cm^{-1}. ^{1}HNMR ($CDCl_3$): 1.79, 2.0 (m, 2H, methylene), 3.8, 3.8 (S, 4H, methylene), 4.9 (m, 1H, methine), 7.5-7.9 (m, 3H, quinoxaline), 7.12-7.95 (m, 20H, Ar-H), 7.9-8.1 (m, 4H, benzimidazole). Mass: m/z: 674.2 (M+).

NVI 5: Yield: 60%, m.p: 119°C, IR (KBr) cm^{-1}: 1770 (C=O str.), 1666 (C=N str.), 1342 (C-N str.), 3037 (Ar-H str.), 3325 (C-H str.) cm^{-1}.^{1}HNMR ($CDCl_3$): 1.8,2.0 (m, 2H, methylene), 3.1,3.8 (S, 4H, methylene), 3.73 (S, 3H, methoxy), 4.9 (m, 1H, methine), 7.5-7.9 (m, 3H, quinoxaline), 6.72-7.9 (m, 19H, Ar-H), 7.06-8.08 (m, 4H, benzimidazole). Mass: m/z: 704.2 (M+) .

NVI 7: Yield: 62%, m.p: 120°C, IR (KBr) cm^{-1}: 3758 (O-H str.) 1770 (C=O str.), 1666 (C=N str.), 1337 (C-N str.), 3037 (Ar-H str.), 3325 (C-H str.) Cm^{-}. ^{1}HNMR ($CDCl_3$): 1.8, 2.0 (m, 2H, methylene), 3.1,3.8(S, 4H, methylene), 4.9 (m, 1H, methine), 5.0 (S, 2H, Ar-OH), 7.5-7.9(m, 3H, quinoxaline), 6.6-7.7(m, 18H,Ar-H), 7.0-8.1 (m, 4H, benzimidazole). Mass: m/z: 706.2 (M+)

NVI 10: Yield: 59%, m.p: 110-111°C, IR (KBr) cm^{-1}: 3750 (O-H str.), 1775 (C=O str.), 1660 (C=N str.), 1335 (C-N str.), 3037 (Ar-H str.), 3325 (C-H str.) Cm^{-1}. ^{1}HNMR ($CDCl_3$): 1.8,2.0 (m, 2H, methylene), 3.1,3.8 (S, 4H, methylene), 3.73 (S, 3H, methoxy), 4.9 (m, 1H, methine), 5.0 (S, 1H, Ar-OH), 7.5-7.9 (m, 3H, quinoxaline), 6.9-7.7 (m, 18H, Ar-H), 7.0-8.0 (m, 4H, benzimidazole).Mass: m/z: 720.2 (M+)

NVI 11: Yield: 69%, m.p: 110-112°C, IR (KBr) cm^{-1}: 753 (C-Cl), 1770 (C=O str.), 1660 (C=N str.), 1335 (C-N str.), 3037 (Ar-H str.), 3325 (C-H str.) Cm^{-1}. ^{1}HNMR ($CDCl_3$): 1.7, 2.0 (m, 2H, methylene), 3.1, 3.8 (S, 4H, methylene), 4.9 (m, 1H, methine), 7.5-7.9 (m, 3H, quinoxaline), 7.0-7.7 (m, 19H, Ar-H), 7.1-8.0 (m, 4H, benzimidazole).Mass: m/z: 708.2 (M+)

NVI 15: Yield: 68%, m.p: 132-124°C, IR (KBr) cm^{-1}: 755 (C-Cl), 1768 (C=O str.), 1660 (C=N str.), 1332 (C-N str.), 3037 (Ar-H str.), 3325 (C-H str.) Cm^{-1}. ^{1}HNMR ($CDCl_3$): 1.8, 2.0 (m, 2H, methylene), 3.1, 3.8 (S, 4H, methylene), 3.73 (S, 3H,methoxy), 4.9 (m, 1H, methine), 7.5-7.9 (m, 3H, quinoxaline), 6.7-7.8 (m, 18H, Ar-H), 7.0-8.1 (m, 4H, benzimidazole). Mass: m/z: 738.2 (M+)

Biological activity

Antifungal Activity

All the compounds were evaluated in vitro for antifungal activity(Sharma,1980) by using cup plate

method by different strains of fungi like *Aspergillus fumigates, Candida Albicans, Candida glabrata* and *candida krusei.* All the compounds along with standard Fluconazole were used at a concentration of 250 mg/ml. 10%DMSO in methanol was used so solvent control and sabour and dextrose agar was used as culture medium. Compounds NVI-13, NVI-14, and NVI-15 exhibited highest degree of antifungal activity.

Antibacterial Activity

This study was assayed by employing the cup plate method(Sharma,1980) by measuring inhibition zones in mm. All the tested compounds along with standard Ciprofloxacin was screened in vitro for antibacterial activity against gram positive *Staphylococcus aureus and Bacillus subtilis,* gram negative *Pseudomonas aeuroginosa* and *Escherichia coli* . The sterile nutrient agar medium was melted and inoculated with 16 –18 hours old broth culture at 1% level. The inoculation has to be completed under aseptic conditions and when the medium was in molten state. The inoculated medium was transferred to sterile Petri dishes, evenly distributed and allowed to solidify. Thereafter the cups (8mm diameter) were made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. Into each of these cups, 0.05 ml (50µg) of the test compound/reference standard was added using a micropipette. The plates were incubated at 37°C for 16 hr and the zone of inhibition was measured. The data indicated that compounds NVI-5, NVI-10, NVI-15 showed excellent activity against *E.Coli* and *Bacillus subtilis*. NVI-14, NVI-15 showed excellent activity against *Staphylococcus aureus.* NVI-14, NVI-15 has shown little activity against *Pseudomonas aeuroginosa.*

3.RESULTS AND DISCUSSION

Synthesis of some phenyl pyrazolo benzimidazolo quinoxaline derivatives (Scheme-I) by condensing benzimidazolo quinoxaline chalcones with different aromatic acid hydrazides have been done successfully. Physical data are shown in (Table-1). The melting point of the compounds were determined on a Thoshniwal electric melting point apparatus and the values were uncorrected. I.R spectra of the compounds were recorded on a Thermo Nicolet Nexus670-FTIR, IICT, Hyderabad using KBr disc method. 1H NMR spectra were recorded onAvance-300, IICT, Hyderabad using $CDCl_3$ as solvent. Mass spectra were recorded on HITACHI RMU GL, IICT, Hyderabad. All the solvents used were of analytical grade. All the synthesized compounds were screened for their anti microbial activities Compounds NVI-13, NVI-14, and NVI-15 exhibited highest degree of antifungal activity (Table-2). Compounds NVI-5, NVI-10, NVI-15 showed excellent activity against *E.Coli* and *Bacillus subtilis*. NVI-14, NVI-15 showed excellent activity against *Staphylococcus aureus.* NVI-14, NVI-15 showed has shown little activity against *Pseudomonas aeuroginosa.* (Table-3)

4.ACKNOWLEDGEMENTS

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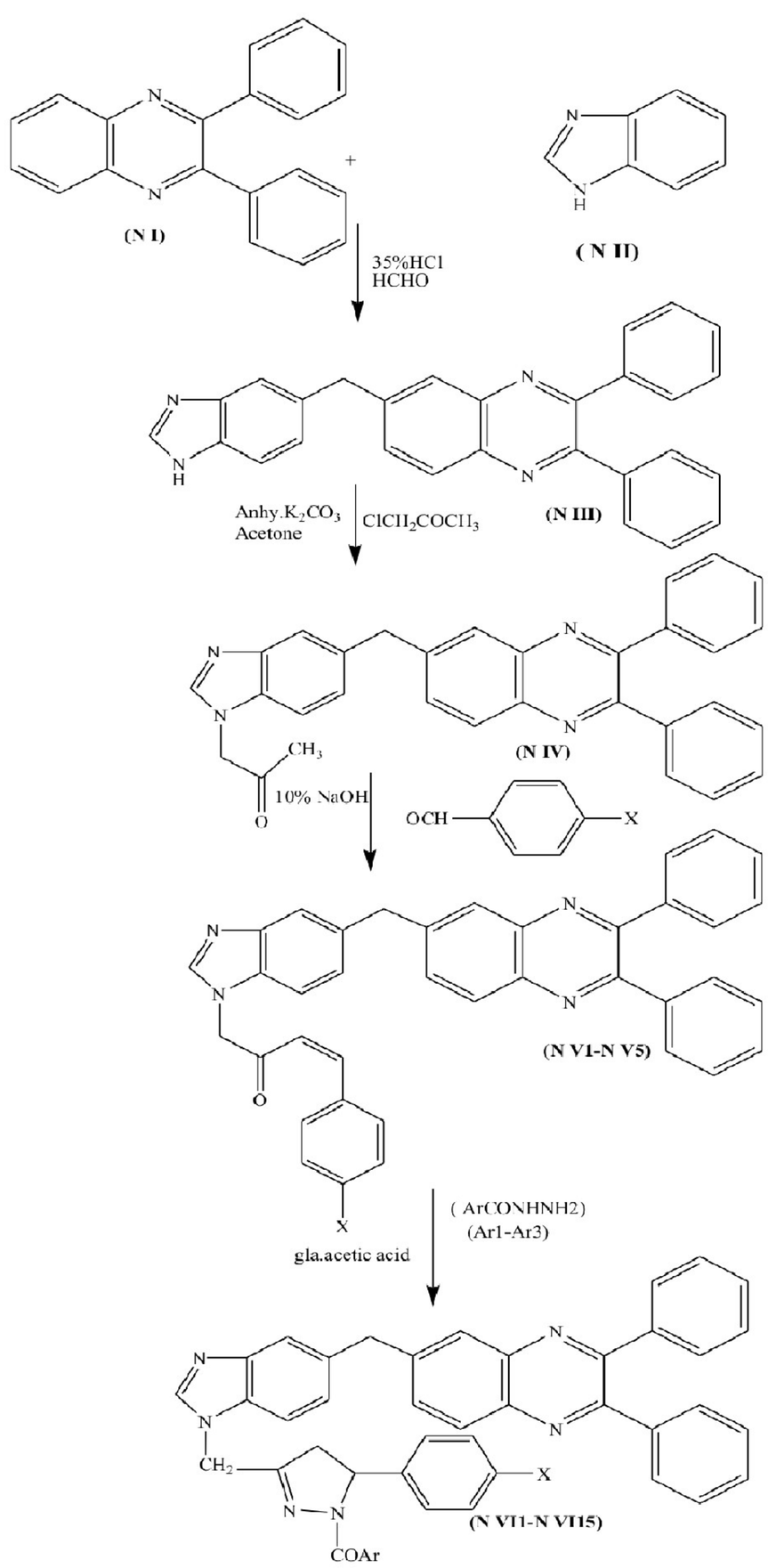

X= H, OH,Cl, F, OCH_3
Ar= $-C_6H_5$, $-C_6H_4OH$, $-C_6H_4Cl$

SCHEME-I

Table – 1. Physical data of phenyl pyrazolo benzimidazolo quinoxaline derivatives

Compd.	X	Ar	Molecular Formula	Melting point range (°C)	% Yield	R_f value
NVI 1	H	C_6H_5	$C_{45}H_{34}N_6O$	122	70	0.8
NVI 2	OH	C_6H_5	$C_{45}H_{34}N_6O_2$	114	67	0.82
NVI 3	F	C_6H_5	$C_{45}H_{33}FN_6O$	112	66	0.8
NVI 4	Cl	C_6H_5	$C_{45}H_{33}ClN_6O$	112	78	0.91
NVI 5	OCH_3	C_6H_5	$C_{46}H_{36}N_6O_2$	116	67	0.8
NVI 6	H	OHC_6H_4	$C_{45}H_{34}N_6O_2$	120	66	0.81
NVI 7	OH	OHC_6H_4	$C_{45}H_{34}N_6O_3$	120	80	0.9
NVI 8	F	OHC_6H_4	$C_{45}H_{33}FN_6O_2$	108	45	0.9
NVI 9	Cl	OHC_6H_4	$C_{45}H_{33}ClN_6O_2$	102	45	0.8
NVI 10	OCH_3	OHC_6H_4	$C_{45}H_{36}N_6O_3$	110	67	0.83
NVI 11	H	ClC_6H_4	$C_{45}H_{33}ClN_6O$	120	56	0.8
NVI 12	OH	ClC_6H_4	$C_{45}H_{33}ClN_6O_2$	120	78	0.82
NVI 13	F	ClC_6H_4	$C_{45}H_{32}ClFN_6O$	131	76	0.80
NVI 14	Cl	ClC_6H_4	$C_{45}H_{32}Cl_2N_6O$	130	56	0.98
NVI 15	OCH_3	ClC_6H_4	$C_{46}H_{35}ClN_6O_2$	123	54	0.81

Table 2- Antifungal activity of phenyl pyrazolo benzimidazolo quinoxaline derivatives

Compound	Antifungal activity [Diameter of the inhibition zone(mm)]			
	Aspergillus Fumigates	*Candida Albicans*	*Candida Krusei*	*Candida Glabrata*
Control	0	0	0	0
Fluconazole	10	28	20	16
NVI 1	-	-	-	-
NVI 2	-	-	-	-
NVI 3	6	16	14	9
NVI 4	6	17	12	9
NVI 5	7	15	13	9
NVI 6	-	-	-	-
NVI 7	-	-	-	-
NVI 8	6	15	10	9
NVI 9	6	18	11	10
NVI 10	5	15	11	9
NVI 11	5	22	9	8
NVI 12	9	22	11	8
NVI 13	10	23	17	15
NVI 14	8	23	18	15
NVI 15	9	28	18	15

Table 3- Antibacterial activity of phenyl pyrazolo benzimidazolo quinoxaline derivatives

Compound	Antibacterial activity [Diameter of the inhibition zone(mm)]			
	Staphylococcus aureus	*Bacillus Subtilis*	*E.coli*	*Psedomonas aeuroginosa*
Control	0	0	0	0
Ciprofloxacin	20	21	20	19
NVI 1	-	-	-	-
NVI 2	10	10	11	9
NVI 3	15	15	15	10
NVI 4	12	15	15	10
NVI 5	15	19	19	-
NVI 6	10	12	-	-
NVI 7	11	13	14	9
NVI 8	14	16	18	8
NVI 9	14	18	19	-
NVI 10	16	20	19	12
NVI 11	10	13	12	8
NVI 12	12	13	14	9
NVI 13	16	11	11	13
NVI 14	20	13	13	14
NVI 15	20	21	20	15

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