Eco-Friendly Synthesis and Biological Evaluation of Novel Quinoxaline Derivatives

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Abstract: *An efficient, mild, and environmentally benign protocol has been developed for the synthesis of quinoxaline derivatives using the uronium salt HCTU as a Lewis acid catalyst. The methodology involves the condensation of various aromatic 1, 2-diamines with phenacyl bromide and its substituted analogues under optimized conditions, affording the desired quinoxaline derivatives in high yields and short reaction times at room temperature. This metal-free catalytic system avoids harsh reaction conditions and hazardous solvents, offering a cost-effective and green alternative to existing synthetic approaches. The synthesized compounds were evaluated for their biological activities and exhibited promising anticancer activity against MCF-7, A-549, and PC-3 cancer cell lines, along with significant antioxidant activity as determined by the DPPH assay. The operational simplicity, excellent yields, and eco-friendly nature of this protocol make it a valuable contribution to the synthesis of biologically active quinoxaline derivatives*

Keywords*:* Quinoxaline, HCTU, 1,2-diamines, anticancer activity

I. INTRODUCTION

Quinoxalines are heterocyclic compounds that include nitrogen; their functional derivatives are known for their wide range of biological uses such as antifungal,[1]anticancer,[2] antiviral,[3]kinase inhibitor,[4] biocide,[5]and anti-HIV activity against anti-HIV IIIB stains.[6]These heterocyclic compounds are also find its importance in the other fields beside biological activity such as pharmaceuticals,[7]sensing of ions,[8-10] dye industries[11], agro-chemicals[12], organicsemiconductors,[13]and chemically controllable switches[14].Looking to the applications of quinoxaline derivatives, enormous methodologies are developed for its synthesis. The most common method for the synthesis relies on the condensation of an aryl 1,2-diamine with a 1,2-dicarbonyl compound,[15]. Similarly, 1,2-keto hydroxyl compounds undergo reaction *via* tandem oxidation procedure involving catalysts such as $Pd(OAc)_2$,[16]MnO_2,[17] I_2/DMSO,[18]. Quinoxalines and its derivatives are also synthesized by oxidative cyclisation of α-haloketones and 1,2-diaminesfrom epoxides,[19]and $CeCl_3.7H_2O$.[20] Some noted catalyst or reagents used for the synthesis of quinoxalines includes CAN,[21] $CuSO_4.5H_2O$,[22] PTSA,[23]TMSCl,[24]*N*-Bromosuccinimide,[25]T3PDMSO or T3P,[26] PEG-400,[27]ZnI_2,[28] $InCl_3$,[29]$Ga(OTf)_3$,[30]$Pd(OAc)_2$,[31]$SbCl_3$,[32]and $NbCl_5$,[33]. The majority of established synthetic routes for quinoxaline and its derivatives exhibit various drawbacks, including the utilization of costly transition metal catalysts, severe reaction conditions, and the incorporation of hazardous or toxic organic solvents, resulting in yields that are only moderate to good. Uronium salt-based coupling agents, such as TBTU, TATU, COMU, and HBPyU, are increasingly utilized in synthetic organic chemistry, for instance synthesis of glycopeptide[34], for esterification[35],and for condensation reaction.[36] Here, our research group found that, the uronium salts can also be used as a Lewis acid catalyst in the synthesis of heterocyclic compounds[37]. Therefore, we intend to report the use of uronium salt (HCTU) as a Lewis acid catalyst to achieve the targeted compounds. In the recent years, catalyst has gained more importance due to enviro-economic factors. They have successfully been utilized in several organic transformations to reduce unwanted waste material. In pursuit of developing efficient and ecologically friendly synthetic techniques utilizing cost-effective and eco-friendly catalysts for organic transformations.[38a-c], we have investigated the synthesis of quinoxalines using HCTU coupling agent as a catalyst.

II. EXPERIMENTAL

GENERAL METHODS

All the chemicals, catalyst and reagents were purchased from Merck. Solvents were distilled before use. The progress of reactions was monitored by thin layer chromatography with TLC Silica gel 60 F_{254} purchased from Merck. Column chromatography was performed on silica gel (60–120 mesh). Melting points were recorded by *an open glass capillary sealed at one end melting point tube and are uncorrected*. The IR spectra were recorded on PerkinElmer Frontier FT-IR spectrophotometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra wererecordedonBruker Ultra shield, Avance II model NMR spectrometer. Chemical shifts of ^{1}H and ^{13}C NMR arereported in parts per million (ppm) from tetramethyl silane (TMS) as an internal standard in$CDCl_3$/DMSO-$d_6$as a solvent. Mass spectra were recorded on AB SCIEX QTRAP 3200 model LC-MSspectrophotometer.

GENERAL PROCEDURE

In 50 mL round bottom flask, 1,2-diamine (1.0 mmol) and phenacyl bromide (1.0 mmol) were stirred at room temperature for 5 minutes followed by addition of HCTU (30 mol%) in 5 mL DMF. The progress of reaction was monitored by TLC. After completion of the reaction,work up was done and the product was extracted with EtOAc thrice (5 mL each), dried over the anhydrous Na_2SO_4, filtered and evaporated under reduced pressure which was purified by silica gel column chromatography (60-120 mesh) using EtOAc-pet ether (5:95) to obtain the pure product.

SPECTARL DATA FORCOMPOUNDS

2-phenylquinoxaline (3a): yellow solid; mp 76-77.5[39a]°C; IR (solid, KBr, ν_{max}, cm^{-1})2920, 2850, 2360, 1541, 1444, 1122, 1076, 954, 669, 549;^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.26-8.04 (m, 4H), 7.77 (m, 2H), 7.63-7.45 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.86, 143.38, 142.32, 141.61, 136.80, 130.28, 130.19, 129.64, 129.54, 129.16, 127.56;

6-nitro-2-phenylquinoxaline (3b): brown solid; 165 -168[39a] °C; IR (solid, KBr, ν_{max}, cm^{-1})2920, 2358, 1521, 1350, 1078, 964, 690; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.50 (s, 1H), 9.03 (d, J = 2.5 Hz, 1H), 8.56 (dd, J = 9.2, 2.5 Hz, 1H), 8.31-8.24 (m, 3H), 7.62 (dd, J = 9.0, 3.2 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.34, 147.47, 145.51, 144.94, 140.37, 135.65, 131.42, 131.20, 129.44, 127.96, 125.69, 123.80;

III. RESULTS AND DISCUSSION

At first, a model reaction was planned between *O*-phenylenediamine**(1)** (1.0 mmol, 0.108 g) and Phenacyl bromide **(2)** (1.0 mmol, 0.199 g) in ethanol (5 ml), after five minutes of stirring at room temperature catalytic amount of HCTU (O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (30 mol%, 0.123g), was added to this reaction mixture, then reaction contents were stirred at room temperature. After 30 minutes, the completion of reaction was confirmed by TLC. Next, the amount of catalyst was kept steady and a variety of solvents screening has been done and examined that, when polarity of the solvent is increases, the yield of the product is also increases with less reaction time(Table 1 entry 1, 3, 4 & 5). In water the obtained yield of the product was very less even after 100 min of stirring at room temperature, this may be due to the insufficient solubility of the starting materials in the water (Table 1, entry 6).

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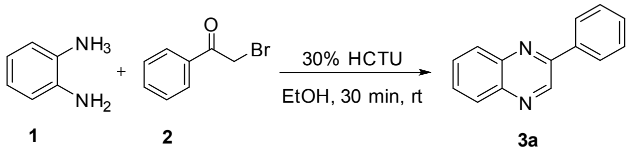
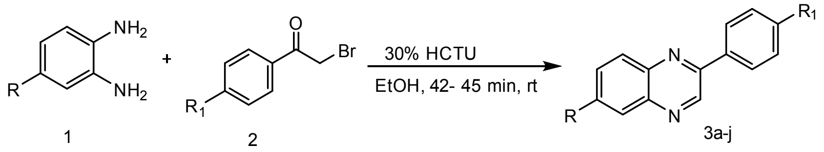


Scheme 1: Model reaction between *O*-phenylenediamine and Phenacyl bromide

From the solvents study, the DMF was found to be an appropriate solvent for this reaction (Table 1, entry 11). Similarly, the effect of % loading of the catalyst was examined in the DMF solvent and observed that, when the percentage of catalyst increases the chemical yield of the reaction also have been increases and required time to accomplish the reaction was decreases (Table 1, entry 7-11).We have observed that there was no clear effect onreactiontime and overall yield of the product when the percentage of catalyst loading amount added more than 35% mol % and 40 mol % respectively (Table 1, entry 12 &13). Therefore, we have finalized the optimized reaction condition for this methodology which was able to give product in 90% yield.

TABLE 1:-SYNTHESIS OF QUINOXALINES DERIVATIVES USING *O*-PHENYLENEDIAMINE AND PHENACYL BROMIDE IN PRESENCE OF HCTU CATALYST



R= -H, -NO_2, -OMe, etc.
R_1= -H, -Cl, -Me, etc.

Entry[3a-j]	1,2, dimine (1)	Phenacylbromide (2)	Product (3a-j)	Time (min)	Yield[b] %
a	R= H	R_1= H		45	90
b	R= NO_2	R_1= H		42	93
c	R= OMe	R_1= H		45	83
d	R= F	R_1= H		42	92
e	R= Cl	R_1= H		45	91

f	R= H	R_1= Me		45	90
g	R= F	R_1= Me		42	94
h	R= H	R_1= Cl		45	90
i	R= F	R_1= Cl		42	94
j	R= OMe	R_1= Cl		48	82

1, 2-diamine**1** (1.0 mmol), various phenacyl bromide **2** (2.0 mmol) was dissolved in 5 ml of DMF and after 42-45 min continuous stirring gets final product with good yield. (3a-j)all the products identified by IR, ^{1}H and ^{13}CNMR (b) Isolated yield of the product.

TABLE 2.REACTION OF 5-BROMO 2, 3-DIAMINOPYRIDE AND 3, 3' DIAMINOBENZIDINE WITH PHENACYL BROMIDE

Entry[3k-l]	1,2, dimine (1)	Phenacyl bromide (2)	Product (3k-l)	Time (min)	Yield[b] %
k				45	90
l				57	88

IV. BIOLOGICAL EVALUATION OF SYNTHESISED COMPOUNDS

ANTI-CANCER ACTIVITY OF THE SYNTHESIZED COMPOUNDS (3f AND 3h).

	Human Breast Cancer Cell Line MCF-7											
	% Control Growth											
	Drug Concentrations (µg/ml)											
Entry	**Experiment 1**				**Experiment 2**				**Average Values**			
	10	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
3f	97.7	93.8	87.9	102.0	86.3	98.5	85.9	72.3	89.0	94.8	88.5	85.4
3h	95.1	100.5	105.5	105.5	85.8	104.8	97.3	86.1	86.9	101.2	100.6	97.5
ADR	**-57.0**	**-56.8**	**-55.5**	**-22.7**	**-63.7**	**-54.5**	**-57.9**	**-35.4**	**-58.0**	**-52.1**	**-52.3**	**-21.1**

	Human Lung Cancer Cell Line A-549											
	% Control Growth											
	Drug Concentrations (µg/ml)											
Entry	**Experiment 1**				**Experiment 2**				**Average Values**			
	10	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
3f	96.2	81.7	75.5	83.4	82.8	83.4	76.5	51.0	86.9	83.8	78.2	69.6
3h	89.5	81.7	89.3	91.0	94.7	94.0	84.0	87.0	88.4	87.3	87.8	89.8
ADR	**-13.4**	**-6.8**	**-41.6**	**-32.1**	**-23.0**	**-7.4**	**-41.4**	**-41.2**	**-17.8**	**-9.1**	**-39.5**	**-35.1**

	Human Prostate Cancer Cell Line PC-3											
	% Control Growth											
	Drug Concentrations (µg/ml)											
Entry	**Experiment 1**				**Experiment 2**				**Average Values**			
	10	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
3f	97.3	94.2	92.5	91.9	107.4	91.9	79.5	79.7	101.4	94.6	85.7	90.4
3h	100.0	93.1	94.1	89.0	110.2	98.0	86.9	79.2	103.0	98.1	90.2	88.4
ADR	**-27.1**	**-31.6**	**-31.6**	**-47.8**	**-30.2**	**-31.2**	**-41.9**	**-46.2**	**-31.6**	**-33.6**	**-37.3**	**-47.0**

ADR = Adriamycin (Reference Standard)

During the screening of anticancer activity of the **3f** and **3h** Adriamycin (ADR) were used as standard drugs. Entry **3f** and **3h** were showed positive average values of the concentration compared with standard, so its concentration is required too much high to kill the cancer cell or to inhibit growth of cancer bacteria and found screened compounds were less active against Human breast cancer cell, Human lung cancer cell and Human prostate cancer cells (Table 2.4).

ANTI OXIDANT ACTIVITY OF (3a to 3c)

Entry	**Structure of the compound**	**Name of the Compound**	**Antioxidant Activity Measured by DPPH Assay**
3a		2-phenylquinoxaline	**54.38%**
3b		6-nitro-2-phenylquinoxaline	**62.45%**
3c		6-methoxy-2-phenylquinoxaline	**56.65%**

Synthesized compounds **3a**, **3b** and **3c** were tested for anti-oxidant activity and found potent active measured by DPPH assay method (Table 2.5).

V. CONCLUSION

In summary, we have successfully developed a simple, efficient, mild, and environmentally benign HCTU-catalyzed protocol for the synthesis of a series of quinoxaline derivatives. The synthesized compounds exhibited promising anticancer activity against human breast cancer (MCF-7), lung cancer (A-549), and prostate cancer (PC-3) cell lines. In addition, these compounds demonstrated significant antioxidant activity as evaluated by the DPPH assay method.Various aromatic 1, 2-diamines reacted smoothly with phenacyl bromide and substituted phenacyl bromides under optimized reaction conditions to afford the desired quinoxaline derivatives in high yields and short reaction times (Table 2). The notable advantages of this methodology include easy handling of the catalyst, excellent product yields, reduced reaction times, and the ability to perform reactions at room temperature. Overall, this green and efficient protocol represents a valuable contribution to the synthesis of biologically active quinoxaline derivatives.

VI. ACKNOWLEDGMENT

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