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Triflate-mediated Synthesis of 3-(4-Methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione and its Antimicrobial Activity.

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Abstract
The synthesis of a novel quinazolinone was accomplished by the reaction of quinazolin-2,4-(1H,3H)-dione with trifluoromethanesulfonic anhydride in triethylamine. The antimicrobial activity was determined against eight microorganisms. The organisms were all susceptible to 3-(4-methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione **3** at the concentrations used. The gram positive organisms showed more susceptibility than the gram negative ones.

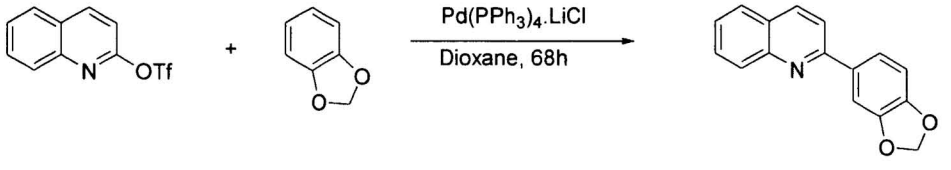
Introduction

A versatile method for carbon-carbon bond formation involves the use of palladium-catalyzed coupling of organic electrophiles with functionalized organostananes. This reaction has many of the attractive features of a general carbon-carbon bond construction method[1,2]. The discovery that vinyl trifluoromethanesulfonates (triflate) undergo the coupling reaction with organostannates paved the way for studies on related reactions of aryl triflate[3,4]. These compounds are valuable starting materials for carbon-carbon bond formation because of their stability.

Trifluoromethanesulfonic anhydride has been used in a broad spectrum of synthetic organic chemistry, ranging from regioselective synthesis of organic and organometallic compounds to carbohydrates, polymer and natural product syntheses. Some dimmers (symmetrical biaryls) have also been isolated in some reaction involved triflates.

Aryltriflate can be easily prepared and have been shown to undergo other reactions other than coupling reaction as show in schemes below. Recent advances have shown tremendously wide applicability for triflic formation and reaction[5,6].

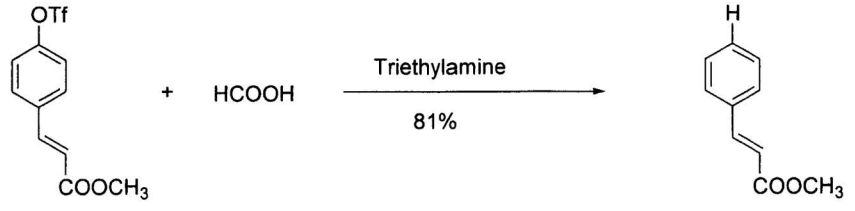
Scheme 1



Tf = CF_3SO_2

There has been a vast development in the chemotherapy of various microbial ailments affecting mankind as well as plants during the last several decades.

Scheme 2



Quinazolinone molecules are known to display a wide range of antimicrobial activity. Some substituted 2-phenyl-3-arylquinazolin-4-ones were synthesized and found to be potent antibacterials[7]. With a view to evaluating the effect of diphenylether group at position 3, the synthesis of 2-methylphenyl-3-[4'-(substituted phenoxy)-phenyl]-6,8-substituted-4-(3H)-quinazolinones was carried out and these compounds were found to be potent against *Bacillus substilis* and *Sarcina lutea*. Some schiff bases of 3-amino-2-methylquinazolin-4-(3H)-ones also exhibited anti-bacterial activity with MIC between 60-100μg/ml. Recently a new dimerization reaction producing quinazoline carbonitriles has also been reported[8]. The synthesis of an unsymmetric dimeric quinazolinone **3** is reported here with its corresponding antimicrobial activity.

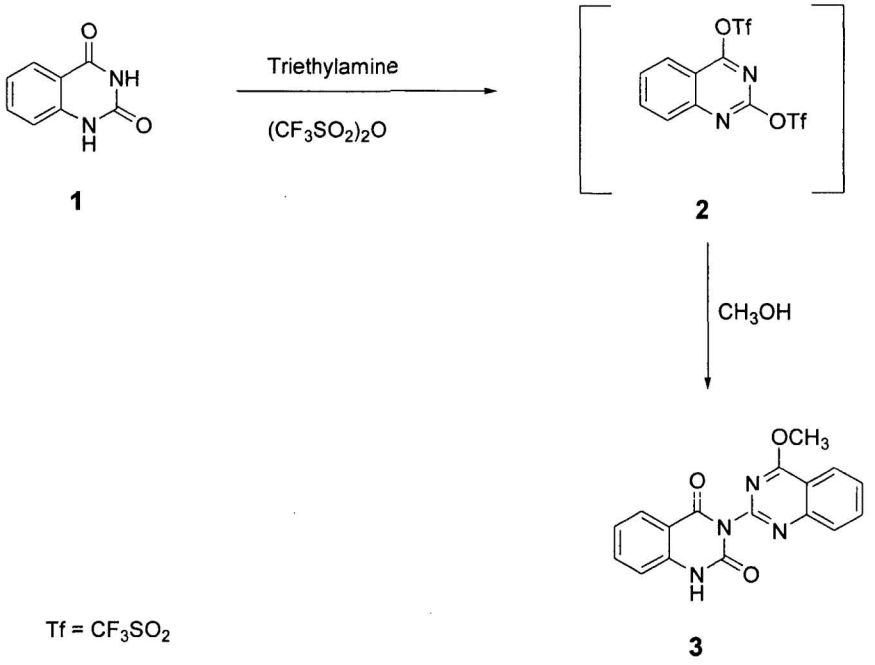
Results and Discussion

Chemistry

The reaction commenced with the aim of synthesizing the triflate **2** of the quinazolinone **1**. The reaction of **1** with trifluoromethanesulfonic anhydride in the presence of triethylamine gave a crude product which was crystallized from methanol. The formation of 3-(4-methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione **3** could have possibly resulted from interaction of methanol with **2** and obviously the participation of the starting material quinazolinone **1**. The possible mechanism for this formation is still under investigation. The compound was unequivocally characterized by elemental and spectroscopic analyses.

The mass spectrum clearly revealed a molecular ion peak (M^+) of 320 which is a strong indication of a dimmer. A methoxy group is shown to be present in the proton NMR at 4.45 ppm and confirmed at in the ^{13}C NMR at 57.2 ppm. Two aromatic regions from the proton NMR are clearly seen. A multiplet at 7.82–7.88 ppm is attributable to hydrogen on carbon 6' while another multiplet due to the two hydrogen on carbon 7' and 8' are centered at 8.12–8.28 ppm. The doublet of doublet at 8.38–8.42 ppm is due to the hydrogen on carbon 5'.

Scheme 3



A triplet centered at 8.50–8.64 ppm contain two hydrogen belonging to carbon 6 and 8. A doublet of a doublet at 9.10 ppm due to the hydrogen on carbon 7 is shown while a doublet of doublet for the hydrogen on carbon 5 is centered at 10.44–10.54 ppm. The hydrogen attached to nitrogen at position 1 is also located at about 10.50 ppm. Additional NMR (NOE and H/H-Cosy) experiments carried out further confirmed the structure of 3-(4-methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione **3**.

Antimicrobial activity

The activity of 3-(4-methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione **3** against some type cultures organisms. *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC 6571 was determined. Clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Salmonella typhymurium* were also determined and the results are shown in the tables 1 and 2. The quinazolinone **3** exhibited antimicrobial activity against the test organisms at different concentrations as demonstrated by the different minimum inhibitory concentrations. It was found out that the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) were the same. There was a very good comparative activity with two known antimicrobial agents at the concentration used. It was also observed that there was lower minimum inhibitory concentrations (MIC) and wider zones of inhibition for the gram positive organisms compared to the gram negative organisms.

Table 1: Minimum Inhibitory Concentration (MIC) of 3-(4-Methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione **3**

Microorganism	3-(4-Methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione (μg)
Escherichia coli NCTC 10418	25
Staphylococcus aureus NCTC 6571 (+)	20
**Escherichia coli*	100
**Staphylococcus aureus*	75
**Pseudomonas aeruginosa*	150
**Klebsiella pneumoniae*	125
**Bacillus subtilis* (+)	75
**Salmonella typhymurium*	200
Dimethylsulphoxide (DMSO)	-

+ Gram positive
*Clinical isolates

Table 2: Antimicrobial Activity

Microorganism	Diameters of Zones of inhibition (mm)		
	3-(4-Methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione (200μg)	Ampicillin (Beecham) (25μg)	Ciprofloxacin (Bayer) (5μg)
Escherichia coli NCTC 10418	22	19	27
Staphylococcus aureus NCTC 6571 (+)	24	15	27
**Escherichia coli*	20	17	26
**Staphylococcus aureus*	23	16	24
**Pseudomonas aeruginosa*	19	-	24
**Klebsiella pneumoniae*	18	12	20
**Bacillus subtilis* (+)	25	14	25
**Salmonella typhymurium*	21	15	22
Dimethylsulphoxide (0.3ml)	-	-	-

+ Gram positive
* Clinical isolates

The organisms were all susceptible to 3-(4-methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione **3** at the concentrations shown in table 2. The gram positive organisms showed more susceptibility than the gram negative organisms. This can be seen in the different zones of inhibition and the minimum inhibitory concentrations in tables 1 and 2 for **3**.

Experimental

Melting points were determined on a Kofler hot stage apparatus and were uncorrected. The ^{1}H and ^{13}C nmr spectra were recorded in appropriate solvent at 200 MHz and 50 MHz respectively with tetramethylsilane as internal reference on a Bruker WM 300 spectrometer. Mass spectra were obtained on a Varian MAT 44S Instrument at 70 ev. The ir spectra were recorded on a Pye Unicam SP3-200 ir spectrophotometer. Silica gel 60 F_{254} (precoated aluminum sheets, 0.2mm thickness; Merck 5549) were used for analytical tlc.

Synthesis of 3-(4-Methoxyquinazolin-2-yl)-quinazolin-2,4-(1H,3H)-dione **3**

Quinazolin 2,4-(1H,3H)-dione (1.72 g, 0.01mol) was weighed into a 250ml two-necked flask and triethylamine (15ml) added and stirred at 0°C for 10 minutes. Trifluoromethane sulfonic anhydride (2.52ml, 0.015mmol) was added respectively and stirred for 5 minutes before allowing to warm up to 25°C. The reaction mixture was then stirred at 25°C for 24 hours. Methanol was then added, stirred and evaporated *in vacuo*. The crude product was crystallized from DMSO–water to afford 3 as colorless needles 2.4g (75%), m.p 176-177°C, IR (KBr): 3100, 3090 (C-H), 1660 (C=0), 1600, 1580 (C=C), 1400, 1030, 790, 740 cm^{-1}, ^{1}H NMR (Acetone-d_6); δ = 4.50 (s, 3H, OCH_3), 7.82-7.88 (m, 1H, H-6'), 8.12-8.28 (m, 2H, H-7',8'), 8.38-8.42 (dd, J=1.3,8.2Hz, 1H-5'), 8.50-8.64 (t, J=7.4Hz, 2H, H-6,8,), 9.02-9.14 (ddd, J=1.3, 7.5, 8.2Hz, 1H, H-7), 10.44-10.54 (dd, J=1.3, 8.2Hz, 2H, H-5, NH); ^{13}C NMR. (Acetone d_6) δ = 57.2 (-OCH_3), 117.5, 125.6, 129.4, 129.7, 130.9, 137.5, 142.6, 142.8, 151.6, 151.8, 171.6; MS m/z (%):=322[M^++2] (21%), 321 [M^++1] (10), 320 [M^+] (16), 319 [M^+-1] (6), 265 (8), 238 (12), 176 (27), 159 (6), 144.0 (18), 119 (21), 92 (18), 79 (75), 64 (100), 52 (69);

Elemental Analysis (320.31) $C_{17}H_{12}N_4O_3$ Cal: C 63.75 H 3.78 N 17.49; Found C 63.70 H 3.60 N 17.42.

Antimicrobial Assay

1ml of overnight cultures of these organisms were sub-cultured into 9 ml Muller-Hinton broth and shaken in a water bath for 4 h at 80 throws per minute and the dilutions made to obtain 10^6 cells/ml. These dilutions were used in testing for the minimum inhibitory concentration (MIC), minimum bacteria concentration (MBC) and in comparison of **3** with known antibacterial agents (ampicillin and ciprofloxacin).

Determination of MIC and MBC:

A stock solution of **3** was made in dimethylsulphoxide (DMSO) to obtain 1mg/ml. Appropriate dilutions of 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200µg/ml in Muller-Hinton broth were made to give a final volume of 2ml in the tubes. One drop equivalent to 0.02ml of 4h old culture of the test organism shaken at 80 throws per minute in a shaker bath at 37°C was inoculated into each of the tubes of the different concentrations of 3 and was incubated at 37°C for 18h. The contents of each of the tubes containing 3 was streaked on a Muller-Hinton agar plate and further incubated for 18h to determine the minimum inhibitory concentration (MIC).

The minimum bactericidal concentration (MBC) was determined by transferring 1ml of the contents of each tube of the appropriate dilutions to 9ml of 0.1% aqueous solution of Peptone (Lablemco) as a diluent. The resulting mixture (0.1ml) was streaked on fresh plates of Muller-Hinton agar, and incubated for 18h to observe and determine any growth.

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