**Synthesis of arylated** ***N*-(4-bromophenyl)-1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxamide by Suzuki–Miyaura cross-coupling reactions and antibacterial activity**

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**ABSTRACT**

In this study, new compounds derived from ciprofloxacin were synthesized by the famous Suzuki reaction. These compounds have been identified and verified by their structural form using IR, 1HNMR, 13CNMR and micro elemental analysis. All these derivatives were tested against different bacteria (*Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Granutice tella adiacens*). The high efficacy results of these derivatives proved significantly greater than the efficacy of the same ciprofloxacin drug.

**KEY WORDS**

Fluoroquinolone, Ciprofloxacin, Antibacterial, DNA grase, Suzuki.

**INTRODUCTION**

Ciprofloxacin is a second-generation fluoroquinolone with a high spectrum of bioavailability as effective antibacterial agents (Akinremi et al., 2012[1]). It is taken orally and has high permeability to tissues and good safety limits (Ahmed et al., 2009[2], Tan et al., 2012[3]). The mechanism of the action of ciprofloxacin is to inhibit the work of topoisomerase II (DNA gyrase) and topoisomerase IV, leading to the closure of bacterial DNA synthesis and this works to stop cell division (Bartzatt et al., 2013[4], Jubie et al 2012 [5]). While inhibition of topoisomerase IV interferes with the function of chromosomal cloning to the particular sibling cell during cell division (Foroumadi et al., 2005[6]). In order to increase the effectiveness of the drug and increase its solubility in water, new compounds were created using the Suzuki-Miyaura. The reaction of Suzuki-Miyaura is a coupling that leads to the formation or appearance of a new carbon-carbon bond. Palladium is introduced as a catalyst and is used extensively in the preparation of polymers, pharmaceuticals and agrochemicals (Schneider et al., 2009 [7], Bandgar et al., 2004[8]). In this reaction, coupling, oxidation and addition of the electrophilic organic group occurs by changing the oxidation by giving the electrons to form a unique Pd-C bond. Transmetalation is added with the addition of a nucleophilic new group of organic compounds that are converted from boron of boronic acid to palladium (Suzuki et al., 1979[9], Cooper et al., 2010[10], Littke et al., 2002[11], Altenhoff., 2004[12]). Boronic acid derivatives are used in this reaction and are available in an environmentally friendly, heat-resistant, inert in air and can recrystallize with alcohol and water (Xu et al., 2014[13], Rekken et al., 2013[14]). Many expensive organic solvents used in this reaction such as tetrahydrofuran as well as costly complex metal catalysts like Pd, Ru, Rh very often allow explanation of the classical reaction methods leading to higher selectivity and increased yields mainly palladium catalyzed C-C coupling reactions are broadly applied in chemical synthesis (Vidossich et al., 2014[15], Mottishaw et al., 2013[16], Ahlquist et al., 2011[17]).

**MATERIAL AND METHODS**

**Instrument and reagents**

Reagents and reactants are purchase d from Sigma-Aldrich company used as procured from commercial suppliers without further purification. Solvents were purified before use. The purity of compounds, and course of reaction were monitored using thin layer chromatography on silica gel-G (Merck grade) with ethyl acetate and hexane mixture as mobile phase, and plates were viewed under UV lamp at 254- 366 nm. The melting points were measured in open capillaries, with the help of (Stuart) melting point (SMP30, England) melting point apparatus, are expressed in οC and are uncorrected. Infrared spectra (IR) were recorded on Shimadzu Prestige-21 Spectrophotometer in Kufa University using potassium bromide (KBr pellets) and the values are expressed in cm-1, 1H NMR and 13C NMR spectra of the compounds were recorded on Bruker (Avance III, Bruker 300 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm in Dmso-d6 as solvent in university of Toronto and elemental analyses were performed on a Flash EA1112 CHN analyzer (Thermo Electron Corporation).

**Chemistry**

*N*-(4-bromophenyl)-1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxamide (**3**) were prepared according to the reported procedures (Nadhir et al., 2016[18]).

***General Procedure for Synthesis of Suzuki Derivatives (4 -17):***

In 25 ml two necked flask was equipped with a magnetic stir bar and 0.0004 mol of (1-cyclopropyl-6-fluoro-*N*-(4-bromophenyl)-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carbox- amide) **(3)** and 0.05gm of Pd(pph3)4 [Palladium tetrakis (tri phenylphosphine)] were added into the flask with 20ml of absolute ethanol under inert atmospheric condition (N2 gas). The mixture was stirred and heated, after that 0.0004 mol of substituted aryl boronic acid and 5ml of solution (5% Na2CO3) were added into the flask. The reaction mixture was stirred and heated at 75°C for (5-6h) then followed by TLC and monitor reactions using (*n*-hexane and ethyl acetate) in 2:3 ratio V/V. The mixture was filtrated off to remove the inorganic salt and the residue of pallidum catalyst, washed by cooled ethanol and the solvent was removed, and decantated by cooled ether.

***1-Cyclopropyl-6-fluoro-N-(2-fluorobiphenyl-4-yl)-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxamide (4)***

From (0.2gm ,0.0004 mol) with 2-fluorophenylboronic acid (0.056gm) Brown; Yield 60%; m.p 118-120oC; FT-IR (KBr): 3433(OH + NHsecondary amine), 1625 (C=O pyridone), 1579 (C=N tautomerism), 1485 (C=C aromatic), 1018 (C-Faryl halide) cm-1. 1H-NMR (DMSO-d6) δ 0.80-1.59 (m, 4H, Hcyclopropyl), 3.0-3.6(m, 8H, Hpiperazinyl), 3.9-4.3(m,1H, Hcyclopropyl), 4.67-4.98 (m, 1H, NHpiperazinyl), 6.41-7.61 (m, 11H, Ar-H), 9.9 (s, 1H, HO-C=Ntautomerism). 13C-NMR (DMSO-*d6*), δ 209 (1C, *C*=Opyridone), 164 (1C, *C*=Ntautomerism), 100-136 (18C, *C*aromatic), 27-31 (2C, *C*piperazinyl), 25 (1C, *C*Hcyclopropyl), 24 (1C,*C*H2cyclopropyl). Anal. Calcu. For (C29H26F2N4O2): C, 69.59; H, 5.24; N, 11.19, Found: C, 69.29; H, 5.16; N, 11.09%.A

**RESULT AND DISCUSSION**

**Chemistry**

Depending on the previous experience in the synthesis of organic compounds in different functional groups in simple reaction with good yields (Najim A. Al-Masoudi et al., 2016), That is why we have been brought up this way for synthesis compounds **(4-16)** moreover aim to evaluated their anti-bacterial activity. The compound **(3)** has been selected as precursor treatment of compound **(3)** with various substituted aryl boronic acid in the presence of Pallidum tertakiss triphenyl phosphine Pd(PPh3)4 as catalyst and 5% of sodium carbonate solution in ethanol and refluxing for (6-8h) afforded compounds **(4-16)** with yield (52-85) %.

Scheme 1. General chart for synthesis compounds **(4-16)**.

Although compound **(3)** contains fluorine and bromine in the origin of the structural formula of the compound, the reaction moves towards the bromine rather than toward the fluorine because the relative activity of the reaction of Suzuki is depending on strong bond C—X and the electronegativity of halogen atom (I > Br > Cl > F) (Norio Miyaura et al., 1995[20]). The reaction happened in ethanol as a solvent (polar solvent) highly soluble, good yield and less time for the reaction. Also used Na2CO3 as a base in this coupling reaction, it is known that the base involved in the coordination sphere of the palladium and the formation of the (Ar-PdL2-OH) from (Ar-PdL2-X) to accelerate the transmetallation step. The most frequently used catalyst, Pd(pph3)4 suffers from this drawback and the phenyl group of the pph3 becomes incorporated in the products giving scrambled derivatives. The bulky phosphine ligand is sufficient to retard this type of side reactions and high yield of the desired product (Nicolaou et al., 2005[21]). Observe through infrared spectrum absorbance of the band at (υ = cm-1) 3200-3414 for NH vibration group of secondary amine and appearance C=O of amide group absorption band at 1720, C=O of pyridone at 1629 in amide derivative **(3)**. all Suzuki compounds give (OH tautomerisum + NH secondary amine) bands at 3360-3452 and appearance the bands of C=O pyridone at the same region in 1624-1629 except the compound **(8)** was shifted to 1631 because this compound contains three fluorine atoms at the same time, and band of C=N tautomerisum of amide group at 1568-1583 except the compounds (**8), (10)** and **(15)** the bands of C=O amide exhibited at 1666,1664,1664 respectively while in compound **(3)** exhibited at 1720 as in figure 1 as example.



Figure 1. Comparison between compounds (**3**) and **(10)** in FT-IR absorption vibration and their structures.

In 1H-NMR spectrum, the presence of amide proton at δ 9.0 ppm in compound (**3)** (amide derivative), all Suzuki derivatives showed a singlet signals in the chemical shift between δ 9.06- 10.2 ppm for proton of (HO-C=N-) tautomerisum except compounds **(8), (10)** and **(15)** exhibited chemical shift at 9.41, 9.11 and 10.0 ppm respectively related to (C=O) amide as well as appeared the band of aldehyde protons for (CHO) group at δ 11.8 and 11.0 ppm in compounds **(14)** and **(16)**. But 13C-NMR of all synthesized derivatives exhibited a clear signal between δ 160- 169 ppm for carbon of imine group (OH-C=N-) tautomerisum except compounds **(8)**, (**10)** and **(15)** appeared a clear signal for carbon of amide in compound **(16)** but the carbon of imine group(OH-C=N-) in compounds **(11)** and **(16)** were shifted to δ 169 and 169 ppm respectively as show in figure 2.



 Figure 2.Resonance of carbon in 13C-NMR and 1H-NMR comparison between compounds **(6)** and **(11)** as well as the tautomerism structures.

There is some important difference in the carbon chemical shift of carbonyl group in pyridone it is between δ 203-212 ppm, derivatives **(10)** and **(15)** exhibited chemical shift between δ 183 and 178 ppm respectively related to carbon of ester group (C=O) so, the compound **(11)** show chemical shift in 60 ppm for methoxy group as well as the derivatives **(14)** and **(16)** exhibited some clear bands at 198 and 202 respectively related to carbon of aldehyde group (CHO). All the synthesized derivatives were tested their solubility in water, the result was increasing the solubility of all these compounds in water compared with solubility of ciprofloxacin drug itself. and all these structures compounds **(2-16)** were also confirmed by (C.H.N.S) analysis, the accuracy of these compounds proved the constant structures.

**Biological evaluation**

An antibacterial activity has been conducted according to piercing method, all ciprofloxacin derivatives **(4**-**16)** were tested by this method against four types of bacteria gram negative such as *Escherichia coli*, *Proteus mirabilis* and gram positive such as *Staphylococcus aureus*, *Granutice tella adiacens*. All derivatives were dissolved in three dissimilar concentrations 0.01 gm, 0.005 gm, 0.001 gm in 10 ml of water, the surface of solid culture media (Nutrient Agar) dried and applied on the plates which had been streaked with standardized bacterial inoculums and incubated at 37 °C for 24h. This technique is based on the determination of an inhibited zone (in mm) proportional to the bacteria in the plates and the results were compared with the antibacterial activity of ciprofloxacin drug.Antibacterial activity was determined by measuring the inhibition zone in mm, the preliminary result show the increasing of the inhibition zone when increasing the concentration of all compounds with all types of bacteria table (3). The results showed that compound (**8**) was the most effective and highest activity against all types of bacteria because this compound contains three fluorine atoms. In particular, compounds (**7), (11)** and (**12)** were found high activity against all types of bacteria because compound (**7)** contains two fluorine atoms, compound (**11)** includes two methoxy groups (2-OMe) and compound (**12)** has two phenyl groups (diphenyl amine) also compounds (**13)** and **(16)** exhibited excellent biological activity towards all tested pathogens bacteria for containing methyl thio group in derivative (**13)** and including thiophen ring in the compound (**16)**. Moreover, all of these derivatives showed high effective even at low concentrations. The results also exhibited that all compounds are effectively much higher than the effectiveness of Ciprofloxacin itself. The compounds (**10)** and (**15**) showed decrease in their activity towards all tested bacteria but the compounds (**4**) and (**14**) were found to be respectable activity against gram-negative (*Escherichia* *Coli, Proteus* *mirabilis)*. Table.3.

  

Table 1.Zone inhibitions (mm) of Ciprofloxacin drug and their derivative **(4-16)** against various microorganisms.

|  |  |  |
| --- | --- | --- |
| Inhibition zone (mm) | Concentrations µg/L | NO |
| *Proteus**mirabilis* | *Escherichia**Coli* | *Granutice tella**Adiacens* | *Staphylococcus**Aureus* |
| 14 | 18 | 22 | 16 | 22 | 25 | 14 | 16 | 18 | 12 | 16 | 20 | 1 0.5 0.1 | **4** |
| 13 | 16 | 20 | 14 | 16 | 23 | 14 | 18 | 21 | 14 | 18 | 21 | 1 0.5 0.1 | **5** |
| 14 | 19 | 22 | 14 | 18 | 22 | 12 | 14 | 18 | `15 | 18 | 22 | 1 0.5 0.1 | **6** |
| 18 | 19 | 21 | 20 | 21 | 23 | 22 | 25 | 29 | 22 | 26 | 28 | 1 0.5 0.1 | **7** |
| 23 | 26 | 29 | 24 | 27 | 31 | 24 | 26 | 30 | 25 | 28 | 32 | 1 0.5 0.1 | **8** |
| 14 | 16 | 18 | 15 | 18 | 19 | 20 | 21 | 23 | 21 | 20 | 22 | 1 0.5 0.1 | **9** |
| 15 | 17 | 19 | 18 | 20 | 21 | 15 | 17 | 19 | 16 | 18 | 20 | 1 0.5 0.1 | **10** |
| 19 | 21 | 25 | 22 | 24 | 26 | 20 | 22 | 24 | 20 | 25 | 27 | 1 0.5 0.1 | **11** |
| 21 | 24 | 26 | 20 | 23 | 25 | 20 |  22 | 26 | 23 | 25 | 28 | 1 0.5 0.1 | **12** |
| 21 | 23 | 25 | 20 | 22 | 24 | 20 | 23 | 25 | 23 | 26 | 27 | 1 0.5 0.1 | **13** |
| 14 | 16 | 18 | 17 | 19 | 20 | 17 | 18 | 21 | 16 | 18 | 20 |  1 0.5 0.1 | **14** |
| 13 | 15 | 18 | 15 | 18 | 20 | 15 | 17 | 19 | 13 | 16 | 18 | 1 0.5 0.1 | **15** |
| 20 | 21 | 26 | 20 | 24 | 27 | 20 | 23 | 25 | 23 | 26 | 28 |  1 0.5 0.1 | **16** |
| 11 | 13 | 17 | 8 | 11 | 14 | 9 | 10 | 12 | 8 | 10 | 13 | 1 0.5 0.1 | **Cip** |

**CONCLUSION**

Synthesized, identification by spectroscopic methods (IR, 1HNMR and 13CNMR) and confirmed all structures by micro-elemental analysis, and evaluated a new series of drug ciprofloxacin derivatives **(4-16)** with their anti-bacterial activity against various microorganisms (*Staphylococcus Aureus*, *Granutice tellaadiacens, Escherichia* *Coli, Proteus* *mirabilis)*. All of these compounds showed high effective even at low concentrations. The results also showed that all compounds are effectively much higher than the effectiveness of ciprofloxacin drug itself.

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[19] **Exploration of the in vitro anti-HIV and cyclin-dependent kinase 2 (CDK2) inhibitory activities of new 6-arylpyrimidines and their nitroso analogues**

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