International Journal of Current Research and Modern Education (IJCRME)
ISSN (Online): 2455 - 5428
(www.rdmodernresearch.com) Volume I, Issue I, 2016

SYNTHESIS AND ANTI HIV1 ACTIVITY OF SOME NOVEL CLUBBED HETROCYCLE COMPOUNDS

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Abstract:

*The present investigation aims to derived several novel 2-methyl-3-(2-(5-phenyl-1H-tetrazol-1-yl) phenyl) quinazolin-4(3H)-one (3) Aromatic Acid Chloride fragment were synthesized to examine their Anti Microbial and Anti **HIV1** activity. 2-methyl-3-(2-(5-phenyl-1H-tetrazol-1-yl) phenyl) quinazolin-4(3H)-one (3) quinazolin-4(3H)-one product tricky. An efficient method was developed for syntheses of novel substituted quinazolinone derivatives by the summarizing of different diamines with benzoxazine reactions demonstrated the benefits of typical reactions; convenient operation, and excellent product yields. These compounds were confirmed by Elemental analysis, IR, ^{1}HNMR, ^{13}CNMR and Mass spectra. Then antimicrobial and anti **HIV1** activities of the sub-stances were tested in-vitro studies. It was found that compound 1-4 possessed a wide range of Anti Microbial and Anti **HIV1** activity (EC_{50}).*

Key Words: Benzoxazine, Biphenyl 4, 4'diamine, Subst.Benzoyl Chloride & Sodiumazide

Introduction:

In spite of these fact that the chemistry quinazolinones has fascinated the attention of investigators for a long time [12, 13, 7] the number of competent approaches to the synthesis of their derivatives containing functional groups is limited [17, 8]. The quinazolinone skeleton appears in many alkaloids, most commonly in the form of 4-(3H)-quinazolinone [16]. The quinazolinone moiety is an important pharmacophore showing many types of pharmacological activities. The quinazolinones are considered to be a privileged structure for drug developments [1, 10, 14]. This has recently inspired the development of a new ring synthesis method. Several successful attempts have been made and recorded in literature demonstrating promising outcomes [15, 3, 11]. The present investigation is a continuation of our earlier [18] study on quinazolinone derivatives. The severe reaction conditions made to the preparation of the quinazolin-4(3H)-one product tricky. Thus, many kind of diverse methods have been developed for the synthesis of quinazolin-4(3H)-one.

Experimental:

General Methods:

Melting points are uncorrected and were recorded on a REMI Series, Lab India Instrument. TLC analysis was done using pre-coated silica gel plates and visualization was done using iodine. IR spectra were recorded in KBr on Schimadzu FT-IR spectrometer. ^{1}H & ^{13}C-NMR spectra were recorded on a Bruker (AC 400MHz) using TMS as an internal standard. Elemental analysis was carried out on a Perkin-Elmer series –II CHNS/O Analyzer 2400. All the chemicals were obtained from Aldrich and all the solvents used were of commercial grade only.

Synthesis of 3-(2-aminophenyl)-2-methylquinazolin-4(3H) one (1)

An equimolar (0.01 mole) mixture of 2-methyl 3, 1 benzoxazine-4-one and biphenyl 4,4'-diamine (0.1 mole) was refluxed for 15 h in the presence of Glac.aceticacid

(10mL). The residue was recrystalized from ethanol and purified by column chromatography to give **1**.

Compound **(1)**: Yield: (74%); m.p. 308°C; IR (KBr, cm^{-1}): 3387.5 (s, NH_2), 3304 (s, N-H), 3085 (s, C-H), 1678.3 (C=O), 1608.68 (s, C=N); ^1H NMR (400MHz, DMSO-d_6, δ / ppm): 8.6 (1H, s, CO-NH C-H), 7.43-7.14 (m,Ar-H), 6.46 (C-H), 2.24 (CH_3); ^{13}C NMR (100MHz, DMSO-*d_6*, δ / ppm): 161 (C1-amide), 155.5 (C-2), 148.2 (N=C), 145.3 (C-8a), 134.5 (C-4"), 127.3 (C-8), 117.2 (C-1"), 24.3 (CH_3).

Synthesis of 4-cholro-N- [4'-(2-methyl-4-oxo-4H-quinazolin-3-yl)-biphenyl-4-yl]-benzamide (2)

The title compound **2** was prepared by using 3-(2-aminophenyl)-2-methylquinazolin-4(3H) one (**1;** 0.01 mole) of an equivalent amount of benzoyl chloride (0.1 mole) was refluxed with pyridine (40 mL) for 11 h. The reaction mixture was cooled, treated with cold ice and neutralized with conc. HCl. The separated solid was filtered and washed with ice cold water. The residue was recrystalized from ethanol and purified by column chromatography.

Compound **(2)**: Yield: (67%); m.p. 171°C; $C_{28}H_{20}ClN_3O_2$; Mol. Wt.: 465.93 Elemental Analysis: C, 72.18; H, 4.33; Cl, 7.61; N, 9.02; O, 6.87; IR (KBr, cm^{-1}): 3471.83 (s, N-H 1°amine), 3303 (s, N-H), 3080.40 (s, C-H), 1680.78 (s, C=O), 1609 (s, C=N), 1530 (C=C aromatic amine), 1270.21 (C-N str 1°aromatic amine), 1170 (s, C-N);^1H NMR (400MHz, DMSO-d_6, δ / ppm): 10.11 (N-H 2°amide), 8.5-8.3 (Ar-CH), 7.84-7.70 (13H, m, Ar-H), 2.13 (CH_3); ^{13}C NMR (100MHz, DMSO-*d_6*, δ / ppm): 161.5 (C1-amide), 165.5 (C-2), 155.5 (C-4), 150 (C-8a), 135.5 (C-1),130 (C-5), 128.6 (C-6), 127.8 (C-8), 122.6 (C-4a), 24.1 (CH_3); MS (*m/z*, (relative abundance, %)): 355.3978 (0.13), 341.4112, 329.0529

Synthesis of 3-{4'[5-(4-chloro-phenyl)-tetrazole-1-yl]-biphenyl-4-yl}-2-methyl-3H-quinazolin-4-one (3)

The title compound **3** was prepared by using 4-cholro-N- [4'-(2-methyl-4-oxo-4H-quinazolin-3-yl)-biphenyl-4-yl]-benzamide (**2;** 0.01 mole) was which taken in a beaker and added a known amount of PCl_5 (0.01 mole) and heated at 100 ^{0}C until the evaluation of HCl fumes ceased. The reaction mixtures contain some unreacted $POCl_3$ which was removed by distillation under reduced pressure. The resulting mixture was treated with ice cold solution of known weight of NaN_3 (0.02 mole), a known volume (40 mL) of acetone, known volume of sodium acetate was added. The reaction mixture was 48h stirred. The acetone was removed by distillation under reduced pressure. The resulting mixture was extracted with $CHCl_3$ then the organic layer was separated and evaporated we got product. The product filtered and washed with ice cold water. The residue was recrystalized from benzene–pet-ether mixture and purified by column chromatography to give **3.**

Compound **(3)**: Yield: (50%); m.p. 254°C; $C_{28}H_{19}ClN_6O$; Mol. Wt.: 490.94 Elemental Analysis : C, 68.50; H, 3.90; Cl, 7.22; N, 17.12; O, 3.26; IR (KBr, cm^{-1}): 3300 (s, N-H), 3060 (s, Ar-C-H), 1658 (s, C=N), 1600 (C=N, tetrazole), 1240 (tetrazole, N-N=N); ^1H NMR (400MHz, DMSO-d_6, δ / ppm): 8.57, 8.6, 8.9, 9.5 (CH,Ar-H), 7.82 (17H,Ar-H,N=C=0), 7.70 (13H,Ar-H,(C=0)N), 2.4 (CH_3); ^{13}C NMR (100MHz, DMSO-*d_6*, δ / ppm): 161 (1-NH). 155.8 (C-2), 140-147.3 (C-8a), 135 (C-4'), 133, 131 (C-3'), 129 (C-6), 127.8 (C-2'), 125.6 (C-8), 123 (C-1"), 121.7 (C-4a), 24.9 (CH_3); MS (*m/z*, (relative abundance, %)): 490.13 (100.0%), 493.13 (10.2%), 492.14 (5.0%), 494.13 (1.8%)

Result and Discussion:

Chemistry

In the present investigation, an attempt has been made to synthesis quinazolinone derivatives through a multi step process. For this purpose, the required

3(2-aminophenyl)-2 methylquinazolin-4(3H)-one **(1)** was prepared according to the literature procedure [5, 4] the condensation reaction between benzoxazin and biphenyl 4,4'-diamine using acetic acid. Formation of the product was confirmed by the formation of intelligent band at 1617cm^{-1} (C=N stretching) along with a peak reading at 1695 cm^{-1} (C=O) in IR spectra. Benzoxazin [6] was converted to 3(2-aminophenyl)-2 methylquinazolin-4(3H)-one **(1)** by nucleophilic substitution reaction with biphenyl 4,4'-diamine, appearance of new peak were observed near 3391.5 cm^{-1} (NH_2 stretching) and 3323.09 cm^{-1} (N-H stretching) also helped in assigning structure of **(1).** When **(1)** was treated with benzoyl chloride [2] in presence of pyridine as a base, nucleophilic reaction took place at the *o*-phenylenediamine site of molecule and as a result quinazolinone ring was formed, to yield a new heterocyclic compound **(2).** New band is observed in the IR spectra at 3084.40 cm^{-1} and a singlet in ^{1}HNMR at δ 2.15 (CH_3) for methylene protons and a singlet at 8.3-8.13 (1H, s, CO-NH C-H) NH proton were in accordance with the structure of quinazolinone ring. Compound (**2**) were condensed with different aromatic acid chloride derivatives. When compound **(2)** was treated with phosporouspenta chloride an intermediate compound was obtained, and then further treated with sodium-azide a compound tetrazole moiety was obtained **(3).** New bands were observed in IR protons, 7.86 (17H, Ar-H, N=C=0) and 8.77 (CH, Ar-H) respectively, confirmed the synthesis of final compounds **(4).** The ^{1}HNMR studies revealed the presence of 1610 cm^{-1} (C=N of tetrazole), 1244 cm^{-1} (tetrazole moiety) compounds at δ 2.43 (CH_3) for methylene protons.

Conclusion:

The present research work demonstrates an unforced and convenient method for synthesizing 3-(2-aminophenyl)-2-methylquinazolin-4(3H)one, 4-cholro-N- [4'-(2-methyl-4-oxo-4H-quinazolin-3-yl)-biphenyl-4-yl]-benzamide and 3-{4'[5-(4-chloro-phenyl)-tetrazole-1-yl]-biphenyl-4-yl}-2-methyl-3H-quinazolin-4-one. The solvent using method for the condensation step proved to be more efficient and friendlier to the environment than the standard procedures. The condensation reaction takes place at relatively high temperatures. This method also simplifies the handling of the reactions and yields quinazolin-4(3H)-one derivative. This procedure is simple, non-toxic and low cost. The reactions scheme exhibited moderate activity and valuable contribution to the existing methodologies. All the biological activities have good inhibition property.

Biological Evaluation:

Report on antimicrobial activity of the given compounds

The antimicrobial activity for the given compounds was carried out by Disc Diffusion Technique (Indian Pharmacopoeia 1996, Vol II A-105). The test micro organisms of Gram positive *Staphylococcus aureus* and Gram negative *Escherichria coli* and fungus *Psudomonous aereus, Basillus substills* were obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient agar and Sabourad dextrose media for bacteria and fungus respectively. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5µg/disc for bacteria and Fluconazole 10 µg/disc for fungi).

Table 1: Antibacterial activity of the quinazolinone compounds at different concentration

S.No.	Name of the Organisms	Zone of inhibition in mm						
		1		2		3		
		50	150	50	150	50	150	STD

		µg	µg	µg	µg	µg	µg	
1.	*Staphylococus aureus*	12	17	11	12	25	20	**30**
2.	*Escherichria coli*	16	19	23	26	20	24	**38**

Standard (STD)- Ciprofloxacin 5 µg / disc for bacteria
Solvent control (Sc)- DMSO

Table 2: Antifungi activity of the quinazolinone compounds at different concentration

S.No.	Name of the Organisms	Zone of inhibition in mm									
		1		2		3					
		50 µg	150 µg	50 µg	150 µg	50 µg	150 µg	STD			
1.	*Psudomonous aureus*	20	21	12	14	12	16	**40**			
2.	*Basillus substilis*	16	20	14	13	13	18	**35**			

Standard (STD) - Ciprofloxacin 5 µg / disc for fungi
Solvent control (Sc) - DMSO

Anti-HIV1 Screening

Cell-Culture

The MT-4 cells were grown in RPMI 1640 DM (Dutch Modification) medium, supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 2 Mm L-glutamine, 0.1% sodium bi carbonate and 20 µg/ mL gentamycin (equal compete medium). The cells were maintained at 37° C in a humidified atmosphere of 5% CO_2 in air. Every 3-4 days and always 2 days before starting the experiment cells were seeded at 3×10^5 cells/ mL. At regular time intervals, the MT-4 cells were analyzed for the presence of mycoplasma and consistently found to be mycoplasma free.

Virus

HIV-1 (Strain HTLV-IIIB/LAI) was obtained from the culture supernatant of HIV-1 infected MT-4 cells lines. The virus titer of the supernatant was determined in MT-4 cells. The virus stocks were stored at 37° C until used.

The present protection achieved by the compound in HIV infected cells was calculated by the following formula:

$$\frac{(OD_T)_{HIV} - (OD_C)_{HIV}}{(OD_C)_{MOCK} - (OD_C)_{HIV}} \text{ expressed in \%}$$

Whereby $(OD_T)_{HIV}$ is the optical density measured with a given concentration of the test compound in HIV- infected cells; $(OD_C)_{HIV}$ is the optical density measured for the control untreated HIV- infected cells; $(OD_C)_{MOCK}$ is the optical density measured for the control untreated HIV- infected cells. The dose achieving 50% protection according to the above formula was defined as the 50% effective concentration (EC_{50}).

Table 3: Anti-HIV1 Activity of compounds

Entry	Strain	IC_{50} (µg/ mL)[a]	CC_{50} (µg/ mL)[b]
1	III_B	>0.456	0.456
2	III_B	>0.482	0.482

3	III_B	>35.1	35.1
AZT	III_B	0.0012	65.9

a - Effective Concentration of compound, achieving 50% protection of MT-4 cells against cytopathic effect of HIV.

b – Cytotoxic concentration of compounds, required to reduce the viability of mock infected MT-4 cells by 50%.

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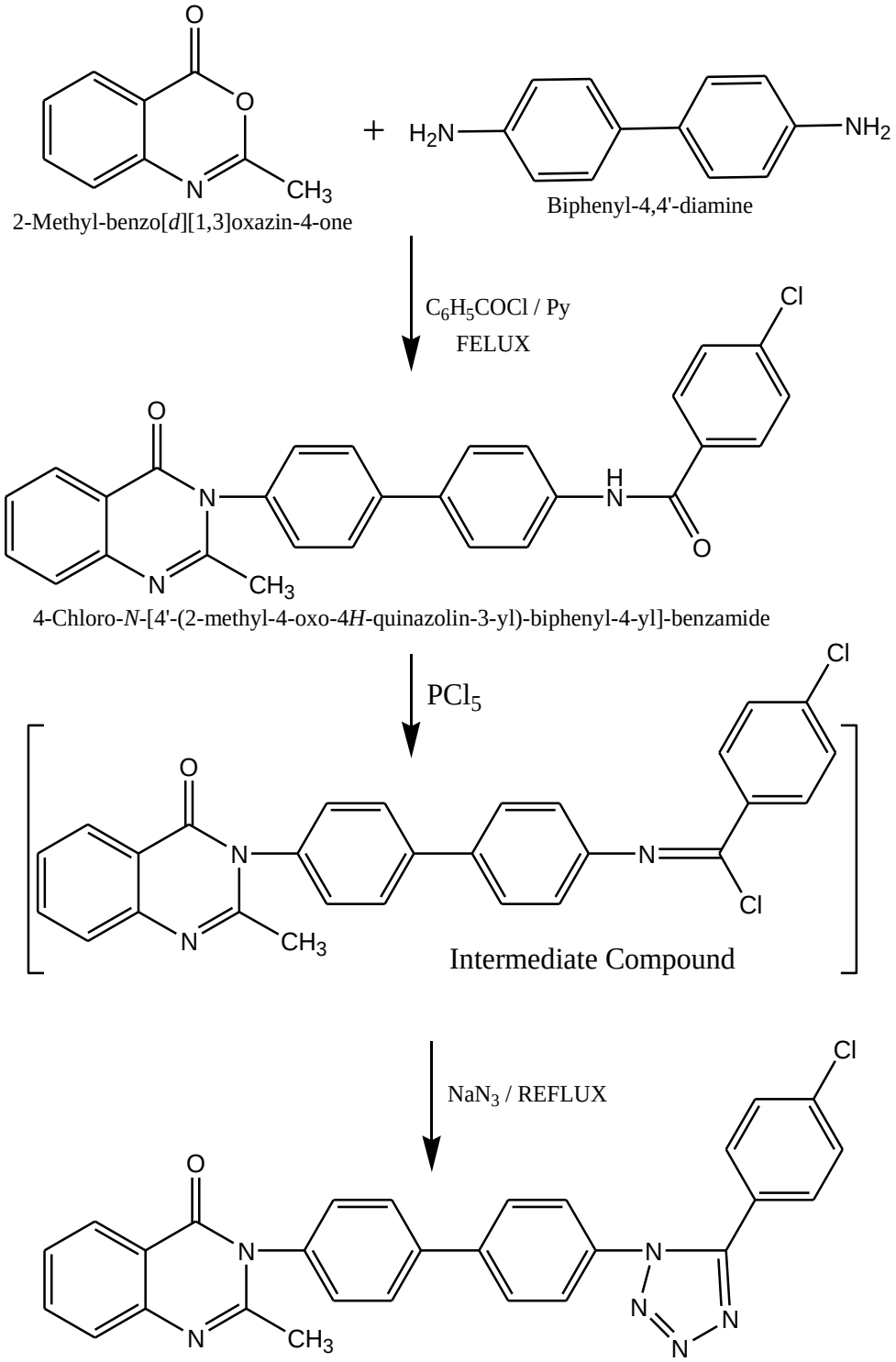
Reaction Scheme



3-{4'-[5-(4-Chloro-phenyl)-tetrazol-1-yl]-biphenyl-4-yl}-2-methyl-3*H*-quinazolin-4-one