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SYNTHESIS AND ANTI-MICROBIAL STUDY OF OXADIAZOLYL INDAZOLES

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ABSTRACT

A rapid and efficient synthesis of substituted **N**-[(1,3,4-oxadiazol-2-yl)methyl]-4-fluoro-6-phenyl-1H-indazol-3-amines has been reported. The key intermediate is 2-(4-fluoro-6-phenyl-1H-indazol-3-ylamino) acetohydrazide (5), which has been obtained by series of reactions starting from cyclisation of 4-bromo-2, 6-difluoro benzonitrile. All the newly synthesized oxadiazole derivatives were screened for their antimicrobial activity.

Key words: 3-Aminoindazole, Suzuki reaction, Oxadiazoles, anti-microbial activity.

1.INTRODUCTION

Since their invention, antimicrobials are one of most significant weapons in fighting bacterial infections. They have extremely benefited the health-related quality of human life. Over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less effective against certain illnesses because of their toxic reactions and due to emergence of microbial resistance. Therefore, it is essential to investigate newer drugs with lower resistance. Indazoles are important class of heterocyclic compounds which have found numerous pharmaceutical applications[1]**.** The indazole ring system represents the core skeleton of an important class of heterocyclic compounds possessing a wide range of biological activities[2]. In particular 3-aminoindazole derivatives attracted great interest due to their activity against a number of pharmacological targets including kinases[3], CNS[4] and inflammatory pathways[5]. Substituted 3-aminoindazoles are used as potent, ATP competitive, reversible inhibitors of AKt[5]. In addition oxadiazoles are the important class of heterocyclic compounds which show wide range of biological activities. The compounds containing 1,3,4-oxadiazole unit currently used in clinical medicine are Raltegravir[6], an antiviral drug and Zibotentan[7], an anticancer agent. US Food and Drug Administration (FDA) approved Raltegravir for treatment of human immunodeficiency virus (HIV-1) infection in combination with other antiretroviral agents in treatment-experienced adult patients who have evidence of viral replication, and HIV-1 strains resistant to multiple antiretroviral agents. Raltegravir is the prototype of a new class of antiretroviral drugs known as integrase inhibitors[8]. Seeking to identify more promising compounds than Raltegravir, in the present investigation we synthesized the oxadiazoles derived from 3-aminoindazoles and studied their antimicrobial activity.

2. MATERIALS AND METHODS

Melting points were recorded using open capillaries and are uncorrected. Analytical TLC was performed on Merck precoated 60 F_{254} silica gel plates. ^{1}HNMR spectra were recorded on

Bruker 300MHz instrument in $CDCl_3$ and $DMSOd_6$ with TMS as an internal standard. Chemical shifts (δ) were recorded in ppm, coupling constants (J) given in Hz.

3. EXPERIMENTAL

3.1 6-bromo-4-fluoro-1H-indazol-3-amine (1)

To the solution of 4-Bromo-2,6-difluoro benzonitrile (2.0g, 9.17 mmol) in ethanol (20 ml) was added hydrazine hydrate (99%) (2.93g, 91.70mmol). Reaction mixture heated in a sealed tube with stirring at 70˚C for 4 hours. Progress of the reaction was monitored by TLC. Reaction mixture concentrated to dryness. The brown coloured solid was purified by crystallisation in ethanol to afford pale yellow needles (1.9g, 90.04%), m p-134˚C, ^{1}HNMR, $CDCl_3$ 4.21 (bs, 2H, $-NH_2$), 7.72 (s, 1H, Ar-H), 7.25(m, 1H, Ar-H), 11.8 (bs, 1H, NH).

3.2 Tert-butyl 3-amino-6-bromo-4-fluoro-1H-indazole-1-carboxylate (2)

To the solution of Compound (1) (1.8g, 5.45 mmol) in dichloromethane (40 ml) was added followed by DMAP (0.66g, 5.45 mmol). Reaction mixture cooled to 0˚C and added boc anhydride (1.20g, 5.45 mmol). Reaction mixture slowly warmed to room temperature and continued the stirring for 15 hrs. Progress of the reaction was monitored by TLC. Reaction mixture was diluted with dichloromethane (50 ml) and washed with water (25 ml*2), brine (25 ml) dried over anhydrous sodium sulphate and concentrated. The crude compound was purified by column chromatography (silica gel, 20-30% ethyl acetate in hexane) to afford gummy solid which solidifies after 2 days (1.6g, 62%), ^{1}HNMR, $CDCl_3$ 1.45 (s, 9H), 4.25 (bs, 2H, $-NH_2$), 7.26 (m, 1H, Ar-H), 7.75 (m, 1H, Ar-H).

3.3 Tert-butyl 3-[(ethoxycarbonyl)methylamino]-6-bromo-4-fluoro-1H-indazole-1-carboxylate (3)

Compound (2) (1.5g, 4.54 mmol) was dissolved in DMF (15 ml) and added anhydrous potassium carbonate (0.62g, 4.54 mmol) and stirred for 30 minutes. Ethyl chloroacetate (0.55g, 4.54 mmol) was added. Reaction mixture stirred at 90˚C for 4 hours. Progress of the reaction was monitored by TLC. Reaction mixture was cooled and quenched with ice cold water (20 ml). Extracted with ethyl acetate (50 ml*3). Ethyl acetate layer washed with water (30 ml*3), brine (30 ml), dried over anhydrous sodium sulphate and concentrated. Crude compound was purified by column chromatography (silica gel 10-20% ethyl acetate in hexane) to afford colourless solid (0.9g, 20.88%) mp-120˚C. ^{1}HNMR $CDCl_3$ 1.32 (t, 3H), 1.43 (s, 9H), 4.0 (s, 2H), 4.7 (bs, 1H), 7.1(s, 1H, Ar-H), 7.7 (s, 1H).

3.4 Tert-butyl 3-[(ethoxycarbonyl)methylamino]-4-fluoro-6-phenyl-1H-indazole-1-carboxylate (4)

Compound (3) (0.8g, 1.92 mmol) was suspended in a mixture of toluene (20 ml) and water (5 ml). Potassium carbonate (0.8g, 5.76 mmol) was added. Reaction mixture degassed with nitrogen for 30 minutes. Phenylboronic acid (0.32g, 2.88 mmol) was added followed by palladium tetrakis catalyst(0.01 g). Reaction mixture stirred at 100˚C for 5 hrs. Completion of the reaction was monitored by TLC. Reaction mixture cooled to room temperature and extracted with ethylacetate (25ml*3). Ethylacetate layer washed with water (25ml*2), brine (25ml), dried over anhydrous sodium sulphate and concentrated. The crude product obtained was purified by column chromatography (5-15% ethyl acetate in hexane) to afford colourless solid (0.5g, 62.11%). ^{1}HNMR 1.33 (t, 3H), 1.40 (s, 9H), 4.12 (s, 2H), 4.75 (bs, 1H), 7.1-7.7 (m, 7H, Ar-H).

3.5 2-(4-fluoro-6-phenyl-1H-indazol-3-ylamino)acetohydrazide (5)

Compound (4), (0.5g, 1.21 mmol) dissolved in ethanol (10 ml) hydrazine hydrate (99%) (80 mg, 2.42 mmol) was added. Reaction mixture was stirred at room temperature for 8.0 hrs. TLC shows completion of the reaction. Reaction mixture concentrated and dissolved in methanol-dichloromethane mixture. Filtered through silica bed. Filtrate was concentrated to get off white coloured solid (0.25g, 83.2%). ^{1}HNMR $DMSOd_6$ 3.02 (bs, 2H), 3.91 (s, 2H), 4.75 (bs, 1H), 7.13-7.76 (m, 7H, Ar-H) 8.53 (bs, 1H) 13.20 (bs, 1H).

3.6 5-[(4-fluoro-6-phenyl-1H-indazol-3-ylamino) methyl]-1, 3, 4-oxadiazole-2-thiol (6)

The mixture of compound (5) (0.3 g, 1.00mmol), Sodium hydroxide (0.04 g,1.00 mmol) and carbon disulphide (0.228g, 3.00mmol) in absolute ethanol

(25 ml) was refluxed for 12 hours. Reaction mixture concentrated under reduced pressure, residue dissolved in water (5 ml), acidified with acetic acid. Precipitate formed was filtered and purified by crystallisation in ethanol to afford colourless solid (0.1g, 29.2%). ^{1}HNMR DMSOd_6, 4.38 (d, 2H), 5.0 (bs, 1H), 7.11-7.65 (m, 7H, Ar-H).

3.7 4-fluoro-6-phenyl-N-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-indazol-3-amine(7)

The solution of compound (5) (0.3 g, 1.00 mmol) in freshly distilled $POCl_3$ (5.0 ml) was refluxed in oil bath for 8.0 hours. Reaction mixture cooled to room temperature, quenched with ice cold water and neutralised with sodium bicarbonate solution. The precipitate formed was filtered, dried and crystallised using ethanol to afford pale yellow compound (0.12 g, 31.0%). ^{1}HNMR DMSOd_6, 4.41 (d, 2H), 5.0 (bs, 1H), 7.21-7.85 (m, 12H, Ar-H) 13.0 (bs, 1H).

3.8 N-[(5-amino-1,3,4-oxadiazol-2-yl)methyl]-4-fluoro-6-phenyl-1H-indazol-3-amine (8)

The solution of compound (5) (0.3 g, 1.00 mmol), cyanogens bromide (0.1 g, 2.00 mmol) in absolute ethanol (25 ml) was refluxed for 24 hours. The progress of the reaction was monitored by TLC. Reaction mixture concentrated in reduced pressure. The residue obtained was dissolved in water and acidified with dilute acetic acid. Precipitate formed was filtered and purified by recrystallisation with methanol to afford pale yellow coloured solid (0.98 g, 32.6%). ^{1}HNMR DMSOd_6, 4.13 (d, 2H), 5.12 (bs, 3H), 7.31-7.65 (m, 7H, Ar-H), 13.0 (bs, 1H).

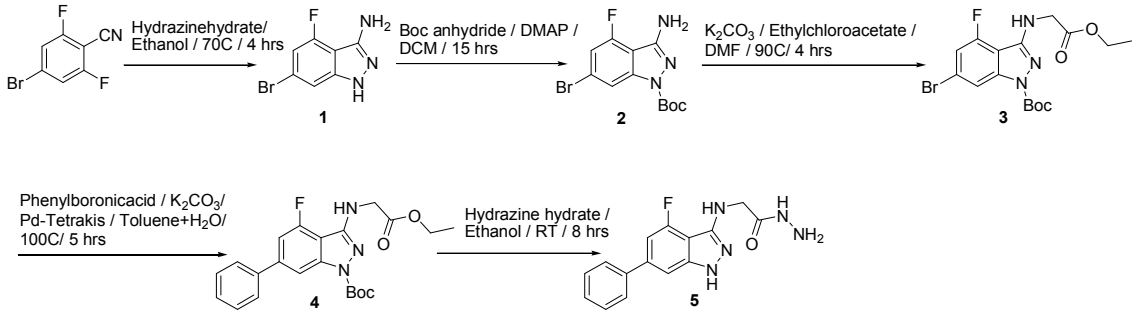
3.9 4-fluoro-N-((5-alky-1,3,4-oxadiazol-2-yl)methyl)-6-phenyl-1H-indazol-3-amine (9)

The solution of compound (5) (0.3 g, 1.00 mmol), triethyorthoformate (0.725 g, 5.00 mmol) in absolute ethanol (25 ml) was refluxed for 18 hours. The progress of the reaction was monitored by TLC. Reaction mixture cooled to room temperature. The crystals obtained were filtered and purified by washing with hexane to afford yellow coloured crystalline solid (0.12 g, 38.8%). ^{1}HNMR DMSOd_6, 4.00 (d, 2H), 5.24 (bs, 1H), 7.01-7.58 (m, 8H, Ar-H), 13.0 (bs, 1H).

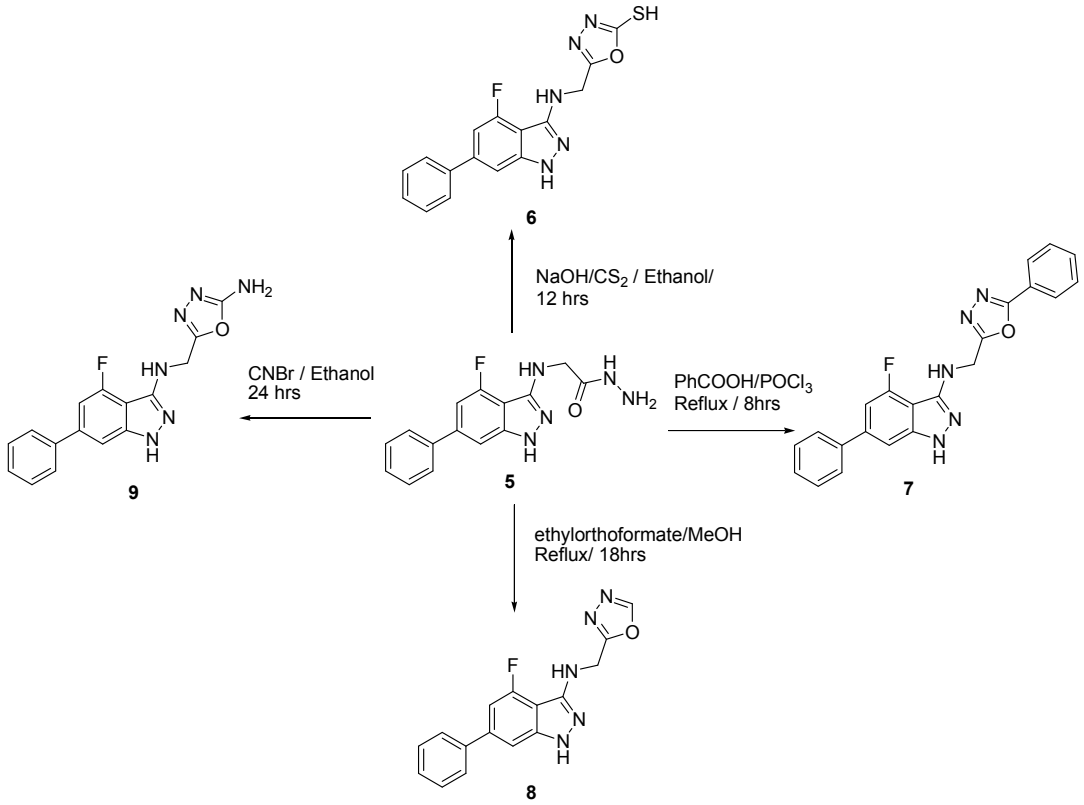
4. RESULT AND DISCUSSION

6-Bromo-4-fluoro-3-aminoindazole (1) was prepared by the reaction of commercially available 4-bromo-2, 6-difluorobenzonitrile with hydrazine hydrate. The ring -NH group was protected with boc using the known literature method[9]. Further boc protected compound (2) was made to react with ethylchloro acetate in the presence of potassium carbonate in DMF to afford tert-butyl 3-[(ethoxycarbonyl)methylamino]-4-fluoro-6-phenyl-1H-indazole-1-carboxylate (3). Compound (3) on reaction with phenylboronicacid under Suzuki conditions resulted in tert-butyl 3-[(ethoxycarbonyl) methylamino]-4-fluoro-6-phenyl-1H-indazole-1-carboxylate (4). 2-(4-fluoro-6-phenyl-1H-indazol-3-ylamino) acetohydrazide (5) was prepared by the reaction of compound (4) with hydrazine hydrate in absolute ethanol. During this reaction the boc protection got cleaved which is confirmed by the absence of sharp peak at δ 1.4 due to methyl protons of the tertiary butyl group. The boc deprotection in presence of bases like hydrazine or ammonia is in well agreement with the literature (scheme-1). The carbohydrazide (5) was used as scaffold to prepare substituted oxadiazoles in next steps using literature methods[10] (scheme-2). Compound (5) on reaction with carbon disulphide in presence of sodium hydroxide in absolute ethanol afforded mercato substituted oxadiazole (6) and compound (5) when refluxed with benzoic acid in phosphorous oxychloride resulted in phenyl substituted oxadiazole (7). Compound (5) further reacted with cyanogenbromide and ethylortoformate to give oxadiazole (8) and 2-amino oxadiazole (9) respectively. All the newly synthesized compounds and intermediates were characterized by NMR spectroscopy.

Scheme-1



Scheme-2



5. ANTI-MICROBIAL ACTIVITY

All the newly synthesized oxadiazole compounds were screened for their anti-bacterial activity against *B.Sbtillis* and *E.Coli* using the standard Ciprofloxacine and anti-fungal activity against *A. Niger* using Grisiofulvin by cup-plate method[11] at the concentration of 1000µg/ml. Compound 6 with -SH substitution at 2nd position of oxadiazole showed 72.2% inhibition against *B.Subtillis* and *E.coli* and same compound showed 50.0% inhibition against *A.Niger*. Compound 7 with phenyl substitution lost complete activity which shows bulkier group at 2nd position of oxadiazole ring will not tolerate. Compound 8 without any substitution at 2nd position of oxadiazole showed 94.4% inhibition against *B.Subtillis* and *E.coli* which is almost equal to the standard and same compound showed 50.0% of inhibiting activity against *A.Niger*. Compound (9) with -NH_2 substitution showed 94.4% inhibition against *A.Niger*. The results are shown in table-1

Table-1
Anti-microbial activity of compounds (6-9).

Compound	B. subtillis		E. coli		A. niger	
	Zone of inhibition mm	Relative inhibition (%)	Zone of inhibition mm	Relative inhibition (%)	Zone of inhibition mm	Relative inhibition (%)
6	19	72.2	19	72.2	15	50.0
7	16	55.5	16	55.5	17	61.1
8	23	94.4	23	94.4	15	50.0
9	15	50.0	15	50.0	23	94.4
DMF	6	-	6	-	6	-
Ciprofloxacin	24	100	24	100	-	-
Grisiofulvin	-	-	-	-	24	100

6. CONCLUSION

Demonstrated the very convenient route for the oxadiazole substituted indazole derivatives and studied their anti-microbial activities by cup plate method compound 8 without any substitution on the oxadiazole ring shown comparable activity with the standard.

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