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Synthesis and Biological Evaluation of Novel 7-Mercaptobenzimidazolyl Fluoroquinolones

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Abstract: The present work is carried out for the synthesis of few novel 7- mercaptobenzimidazolyl fluoroquinolones. The structures of the synthesized compounds were established on the basis of spectral and analytical data. The antimicrobial activities of newly synthesized compounds were evaluated against a number of microorganisms by using Levofloxacin as reference standard. Many of the evaluated compounds were found to exhibit remarkable antibacterial activity and excellent antifungal activity.

Keywords: Mercaptobenzimidazolyl fluoroquinolones, antimicrobial activity.

Introduction:

The fluorinated quinolonesare extensively used in medicinal chemistry due to their potent antibacterial activity against wide varieties of Gram positive and Gram negative bacteria with minimum toxic side effects[1-3]and are hence commonly prescribed antibiotics. Fluoroquinolones exhibit various Pharmacological properties such as antimicrobial[4], anti-inflammatory[5], analgesic[6]and antiviral activities[7]. Fluoroquinolones have also been incorporated in a wide variety of therapeutically interesting antibacterial drugs such as Ciprofloxacin, Levofloxacin,Moxifloxacin, etc. A survey of the literature indicates that substitution and chemical manipulation at position 7 of the fluoroquinolone ring system provides potent antibacterial agents with enhanced biological activities.

The Benzimidazole is one amongst such important nitrogen heterocycles group as several of its derivatives have pharmacological properties and have been marketed as commercial products[8-9]. The mercaptobenzimidazole and its derivatives are a promising class of the bioactive heterocyclic compounds that exhibit a wide range of medicinal uses. They have been reported to be associated with interesting pharmacological properties which include anti-cancer[10], anti-microbial[11], anti-viral[12] and anti- fungal activity[13].

Keeping in view the pharmacological importance of both fluoroquinolone and mercaptobenzimidazolyl moieties, it was thought worthwhile to develop a method for the rapid synthesis of novel fluoroquinolone derivatives containing a mercaptobenzimidazole moiety at position 7.

The present chapter deals with the synthesis and biological evaluation of novel 7-mercaptobenz imidazolyl fluoroquinolones.

Experimental

Melting points were determined using Thermonik melting point apparatus (Campbell Electronics, India) by open capillary method and are uncorrected. Reactions were monitored by thin layer chromatography (TLC) using aluminium sheets coated with silica gel 60 F254 (Merck) in UV chambers. IR spectra were recorded on a Perkin-Elmer 1700 spectrometer in KBr discs. ^{1}H NMR and ^{13}C NMR were recorded at 300MHz in DMSO-d6 using Joel instrument (Joel, Japan). Chemical shifts were measured at δ units (ppm) relative to Tetramethylsilane (TMS). Electrospray ionization mass spectra (ES-MS) were recorded on Varian 300 MS-spectrometer. Elemental analysis data were obtained by employing a Perkin- Elmer 240c analyzer. Solvents were of reagent grade and were purified, dried by standard procedure.

Synthesis of Compound 4a from 1:

To a mixture of acetic anhydride (500 ml), Zinc chloride (2.5g, 2.5 % w/w) and boric acid (27.4 g, 443 mmol), compound **1** (100 g, 338 mmol) was added and the reaction mass was heated to 120-125°C, maintained for 5h. The reaction mass was cooled and the excess of acetic anhydride was distilled out under reduced pressure. Toluene (300 ml) was added and stirred for 1h at 25-30 °C. The precipitated compound was filtered to get borate complex as wet solid. The borate complex was immediately dissolved in acetonitrile and DMF mixture (500:50 ml), added the compound **2**(76.15 g**,** 507 mmol) followed by triethylamine (102.4 g, 1014 mmol) and maintained the reaction for 8h at 25-30 °C. The progress of the reaction was monitored by TLC (disappearance of starting material). The reaction mixture was added to crushed ice (500g) and the pH was adjusted to 1.0-2.0 with hydrochloric acid. The precipitated compound was filtered and dried. The obtained product was recrystallized from methanol to afford compound **4a**.

(S)-10-((3S)-10-((1H-benzo-2yl)thio)-9-fluoro-3-methyl-1-7-oxo-3,5,6,7-tetrahydro-2H[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid(4a)

Yield:76%;Whitesolid; mp:216.4°C-219.3°C;IR(KBr, cm^{-1}) 3395(N-H),1721(C=O),1608(C=O); ^{1}H NMR (DMSO-d_6): δ 1.43 (t,3H,CH_3), 4.45-4.64(m,2H,CH),4.97-5.04(m,1H,CH),7.28-7.31(m,2H,Ar-H),7.52-7.55 (m,2H,Ar-H), 7.78-7.81 (d,1H,Ar-H), 9.11 (s, 1H, olefinic), 12.5 (s, 1H, COOH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6): 17.8 (CH_3), 54.9 (CH), 69.3(CH_2),102.8(CH), 108 (C), 109.6 (C), 113.8 (CH), 122.3 (CH), 123.5 (C), 130.2(C),132.4 (C), 136.2 (C), 146.7(CH), 158 (C), 165.5 (C=O, acid), 176.7 (C=O, ketone) ; Mass (ES): *m/z* 412 $[M+H]^+$; Anal. Calcd. For $C_{20}H_{16}FN_3O_4S$: C, 58.39; H, 3.43; F 4.62; N, 10.21; O, 15.56; S 7.79. Found: C, 58.35; H, 3.42; F, 4.60; N, 10.20; O 15.55;S 7.78.

The same procedure wasfollowed for the preparation of other compounds **4b-4e**.

(3S)-9-fluoro-10-((5-methoxy-1H-benzo[d]imidazol-2yl)thio)-3-methyl-7-oxo-3,5,6,7-tetrahydro-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4b)

Yield: 80%; pale yellow solid; mp:242.1°C - 247.8°C;IR (KBr, cm^{-1}) 3410(N-H),1721(C=O),1608 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.43 (d, 3H,CH_3), 3.77(s,3H,OCH_3), 4.45-4.64 (m,2H,CH_2), 4.99-5.01(m,1H,CH), 6.90-6.93(m,1H,Ar-H), 7.00-7.01(d,1H,Ar-H), 7.41-7.44 (d, 1H,Ar-H), 7.77-7.80(d,1H,Ar-H), 9.11 (s, 1H, olefinic);^{13}C NMR (DMSO-d_6): 18.27 (CH_3), 55.4(CH), 56.1(O-CH3),69.78(CH2),96.67 (CH), 103(C), 107.9(C), 108.46(C), 114.3(CH),114.98(C), 125.0(C),128.6 (C), 129.7(C), 131(C), 145.74(C), 147.1 (CH), 149.1(C-F),157.4(CH),165.9(C=O, acid), 176.8 (C=O, ketone) ; Mass (ES): *m/z* 442 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{16}FN_3O_5S$: C, 57.14; H, 3.65; F 4.30; N, 9.52; O, 18.12; S 7.26. Found: C, 57.16; H, 3.63; F, 4.32; N, 9.52; O 18.14; S 7.27.

(S)-10-((5-amino-1H-benzo[d]imidazole-2-yl)thio)-9-fluoro-3-methyl-7-oxo-3,7-dihydro-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (4c)

Yield: 65%; White solid; mp:293.5°C-298.6°C;IR (KBr, cm^{-1})) 3445(N-H),1724(C=O),1623(C=O); ^{1}H NMR (DMSO-d_6): δ 1.45-1.47 (d, 3H,CH_3), 4.46-4.70 (m,2H,CH_2), 5.0-5.02(m,1H,CH), 7.79-7.86 (m, 4H,Ar-H), 9.09 (s, 1H, olefinic), 14.83 (s, 1H, COOH, exchangeable with D_2O);^{13}C NMR (DMSO-d_6): 17.95(CH_3),55.08(CH),69.05(CH_2),97.8(CH),103.6(C),103.85(CH),107.89(C),110.2(CH),115.7 (CH), 122 (C), 125.2 (C), 127.8(C), 132.2(C),138.6 (C),144.6(C),147.27(CH),160.2(C), 165.75 (C=O, acid), 176.64 (C=O,

ketone) ;Mass (ES): *m/z* 427 $[M+H]^+$; Anal. Calcd. for $C_{20}H_{15}FN_4O_4S$: C, 56.33; H, 3.55; F 4.46; N, 13.14; O, 15.01; S 7.52. Found: C, 56.35; H, 3.56; F, 4.42; N, 13.10; O 15.06; S 7.50.

(S)-9-fluoro-3-methyl-10-((5-methyl-1H-benzo[d]imidazole-2-yl)thio)-7-oxo-3,7-dihydro-2H-[1,4]oxazino[2,3,4-ij]quinolone-6-carboxylic acid(4d)

Yield: 74%; pale yellow solid; mp:241.3°C-247.8°C;IR (KBr, cm^{-1}) 3391(N-H),1745(C=O), 1605(C=O); ^{1}H NMR (DMSO-d_6): δ 1.42 (d, 3H,CH_3), 2.48-2.50(m,3H,CH_3-Ar), 4.45-4.63 (m,2H,CH_2), 4.99-5.01 (m,1H,CH), 7.07-7.15(d,1H,Ar-H), 7.33-7.44 (m, 2H,Ar-H), 7.78-7.81(d,1H,Ar-H), 9.11 (s, 1H, olefinic); ^{13}CNMR (DMSO-d_6): 17.8 (CH_3),22(CH3), 55.4 (CH), 69.4(CH_2),105.3(CH), 109.2(C), 111.4 (C),115.9(CH), 124.9(C), 125(C), 128.9(C),130.2 (C), 132 (C), 134.23(C), 145.65(C), 148.9(C),166.6 (C=O, acid), 176.5 (C=O, ketone) ; Mass (ES): *m/z* 426 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{16}FN_3O_4S$: C, 59.29; H, 3.79; F 4.47; N, 9.88; O, 15.04; S 7.54. Found: C, 59.30; H, 3.76; H, F, 4.45; N, 9.83; O 15.04; S 7.52.

(S)-10-(5-chloro-1H-benzo[d]imidazol-2yl) thio-9-fluoro-3-methyl-7-oxo-3,7-dihydro-2H-[1, 4] oxazino [2, 3, 4-ij] quinolone-6-carboxylic acid (4e)

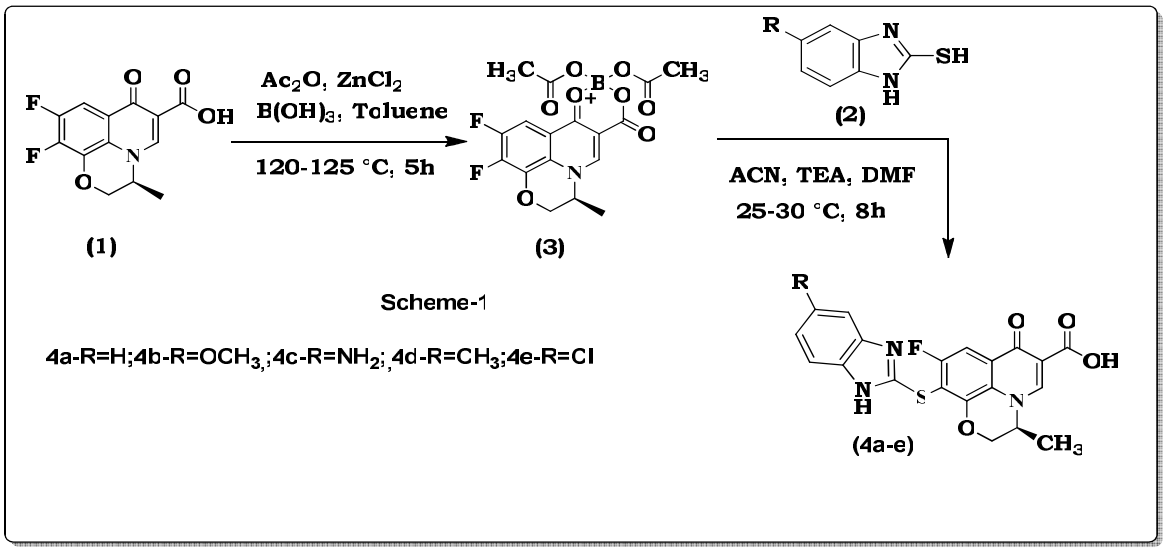
Yield: 68%;White solid; MP:241.4°C-246.3°C;IR (KBr, cm^{-1}) 3418(N-H),1697(C=O),1606(C=O); ^{1}H NMR (DMSO-d_6): δ 1.42(d, 3H,CH_3), 4.40-4.61 (m,2H,CH_2), 4.98-5.00 (m,1H.CH), 7.16-7.19(d,1H,Ar-H), 7.41-7.49 (m,2H,Ar-H), 7.76-7.79(d, 1H, AR-H),9.10 (S, OlefinicH); ^{13}C NMR (DMSO-d_6): 17.84(CH_3), 54.96 (CH), 69.24CH_2),98.5(CH),107.9(C), 108.5 (C), 115.1 (CH), 122.8 (C),124.4(CH), 127.8 (C), 130.2(C),132.4 (C), 136.4(C),138.9(C),146.7(CH), 148.3 (C),149.2(C), 160.7(C),165.5 (C=O, acid), 176.5 (C=O, ketone); Mass (ES): *m/z* 446 $[M+H]^+$; Anal. Calcd. for $C_{20}H_{13}ClFN_3O_4S$: C, 53.88; H, 2.94; Cl, 7.95; F 4.26; N, 9.42; O, 14.35; S 7.19. Found: C, 53.86; H, 2.95; Cl, 7.93, F, 4.23; N, 9.45; O 14.36; S 7.20.

Invitro antimicrobial activity was carried out using disc diffusion assay (Indian Pharmacopoeia 1996,Vol II, A-1 05). Whatman no.1 filter paper discs of 5mm diameter were sterilised by autoclaving for 15 min at 121°c. The sterile discs were impregnated with the test compounds (100µg and 500µg/disc). The agar plates were then innoculated with standard inoculum (10^5 cells/mL broth) of the test organisms namely Staphylococcus aureus (NCIM 2079), E.Coli (NCIM 2065), Pseudomonas Aeruginosa (NCIM 2036), Bacillus subtilis (NCIM 2063), Aspergillus niger (NCIM 105) and Candida albicans (NCIM 3102). They were all obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient agar and Sabouraud dextroseagar medium.Theimpregnated discs were innoculated at 5°Cfor 1h to permit good diffusion and then transferred to an incubator at 37°C for 24 hr. The diameter of inhibition zone was measured using a calibre to the nearest mm. Levofloxacin 5µg/disc for bacteria and Nystatin 100µg/disc for fungi were used as standard.

Results and Discussion:

The focus of the present investigation is on the development of a few *N*-substituted 7-(4-(mercaptobenzo[*d*]imidazol-2-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydro quinolone-3-carboxylic acid compounds **4(a-e**) starting from compound **1**, i.e., 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. Initially the reaction of 1-cyclo-propyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(1)** with 2-mecaptobenzoimidazole (**2)** was carried out in various solvents like acetonitrile, DMF or DMSO by deploying bases like pyridine, triethylamine or potassium carbonate. Almost all attempts failed to give the complete conversion of starting material into desired product with acceptable quality and quantity.

Later, the above condensation reaction was performed using the borate complex protocol which furnished desired product with excellent yields. 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydro quinoline-3-carboxylic acid (**1)** was reacted with boric acid in acetic anhydride in presence of zinc chloride gave the corresponding borate complex (**3),** Further Compound**3** on reaction withsuitably substituted 2-mercaptobenzo[d]imidazoles (**2)** in presence of triethylamine in acetonitrile resulted **4**regioselectively withgood yield. **(Scheme-1)**



During the course of reaction it was observed that the borate complex was unstable and should be used immediately after the filtration.

The structures of the compound 4a-e wereassigned on the basis of their IR (KBr) spectrum, ^{1}H &^{13}C-NMR spectrum (DMSO-d_6) and mass spectrum.

Biological Activities

Antibacterial activity: The synthesized compounds **4(a-e)** were tested for antibacterial activity against Gram-positive organisms *viz. Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079) andGram-negative organisms *viz Escherichia coli* (NCIM 2065), *Pseudomonas aeruginosa* (NCIM 2036)by disc diffusion method recommended by National Committee for Clinical Laboratory (NCCL) standards.Levofloxacin was used as a reference standard**.** It was found that all the newly synthesized compounds were potent against all the tested strains.

Anti-fungal activity: *In vitro* antifungal activity of newly synthesized compounds was studied against the fungal strains Candida*albicans* (NCIM 3102) and Aspergillus*niger*(NCIM 105)by Disc DiffusionMethod.

The minimum inhibitory concentration (MIC) values are presented below in **Table 1.**

All the synthesised compounds exhibited antibacterial activityand were found to have an excellent antifungal activity against A.niger and C.albicans.

Table1: Minimum inhibitory concentration (MIC) values of compounds **4a-e.**

Compound	S.Aureus		E.Coli		P.Aeruginos		B.Subtilis		A.niger		C.Albicans	
Name	500	1000	500	1000	500	1000	500	1000	500	1000	500	1000
4a	18	26	25	36	16	22	16	26	20	22	16	20
4b	23	25	22	32	22	25	18	26	18	20	20	30
4c	20	28	22	30	14	16	30	42	17	20	16	20
4d	22	28	24	30	16	25	18	20	14	20	20	25
4e	17	23	18	24	16	20	16	26	14	20	20	26
STANDARD	26	38	32	40	28	32	33	36	12	18	20	23

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