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Synthesis of some new fluorine containing 1,3,4-oxadiazole derivatives as potential antibacterial and anticancer agents[§]

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The synthesis of a number of 1,3,4-oxadiazole derivatives have been described. 2,4-Dichloro-5-fluorobenzoyl hydrazine **1** on reacting with aromatic acids in presence of phosphorus oxychloride affords 2,5-disubstituted-1,3,4-oxadiazoles **2a-j**. Aroyl hydrazine on reacting with carbon disulphide under basic condition gives oxadiazol-2-thione **3**. Oxadiazol-2-thiones are regioselectively aminomethylated to give *N*-aminomethylated products **4a-c**. Further, **3** undergoes regioselective alkylation on treating with alkyl/aroyl halides to give *S*-alkyl/aroyl products **5a-c**. All the newly synthesized compounds have been screened for their antibacterial activity. Most of them show promising antibacterial activity. Also two out of nine compounds show anticancer activity in the primary anticancer assay.

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2,5-Disubstituted 1,3,4-oxadiazole derivatives possess broad spectrum of pharmacological activities such as antibacterial[1], antifungal[2], antimalarial[3], anticonvulsant[4], anti-inflammatory[5-6] and anticancer[7] activities. 5-Substituted-1,3,4-oxadiazole-2-thione and their derivatives possess CNS depressant[8], pesticidal[9-10], antitubercular[11] and tyrosinase inhibition[12] property. Most widely used fluorine containing antibacterial agents such as norfloxacin and ciprofloxacin are orally active, have broad spectrum and favourable pharmacokinetic and safety profiles[13]. Recent importance of fluorine containing compounds in general and heterocycles in particular have initiated active research in fluorine containing heterocycles. Such compounds have high thermal stability, increased solubility and higher biological activity[14-15]. We therefore are interested in exploring the biological activity of such molecules through structural modifications.

Several 2,5-disubstituted-1,3,4-oxadiazole derivatives[16] are synthesized by cyclization of aroylhydrazine derived from the reaction of 2,4-dichloro-5-fluorobenzoic acid and carboxylic acids (RCO_2H) acid in presence of phosphorus oxychloride (**Scheme I**). Aroyl hydrazine on treating with carbon disulphide under basic conditions gave 1,3,4-oxadiazol-2-thione, which underwent *N*-aminomethylation reaction on treating with formaldehyde and a secondary amine. The alkylation of 1,3,4-oxadiazol-2-thione **3** gave regioselectively *S*-alkylated product[17] (**Scheme II**). All the compounds were characterized by analytical and spectroscopic methods.

In the IR spectrum of compound **2b** showed absorption peak at 3075 cm^{-1} assigned to aromatic C-H stretch. Another peak at 2842 cm^{-1} was due to the presence of OCH_3 group. The peaks for C=N was observed at 1650/1616 cm^{-1}. The peaks at 1451 and 1267 cm^{-1} were assigned to C-O-C and C-O stretch respectively. The medium absorption at 1118, 923/894 was due to the presence of C-F and C-Cl bonds. The ^1H NMR spectrum of compound **2b** showed a doublet at δ 8 (J= 8.7 Hz) assigned to aromatic protons. A sharp singlet was observed at δ 7.4 was assigned to aromatic proton of 2,4-dichloro-5-fluorophenyl moiety. Two distinct doublets at δ 7.05 and 7.03 (J= 8.7 and 8.5) integrating for four protons were assigned to protons of 4-methoxyphenyl moiety. Methoxy protons appeared as sharp singlet, integrating for three protons at δ 3.9. The low resolution EI mass spectrum of compound **2b** showed molecular ion peak at m/z: 337(4%) confirming its formation. This underwent fragmentation to give peaks at m/z: 305 (10%), 215 (26%), 135 (100%) and 77 (24%) respectively. The spectroscopic data for some of the compounds are provided under **Table I**.

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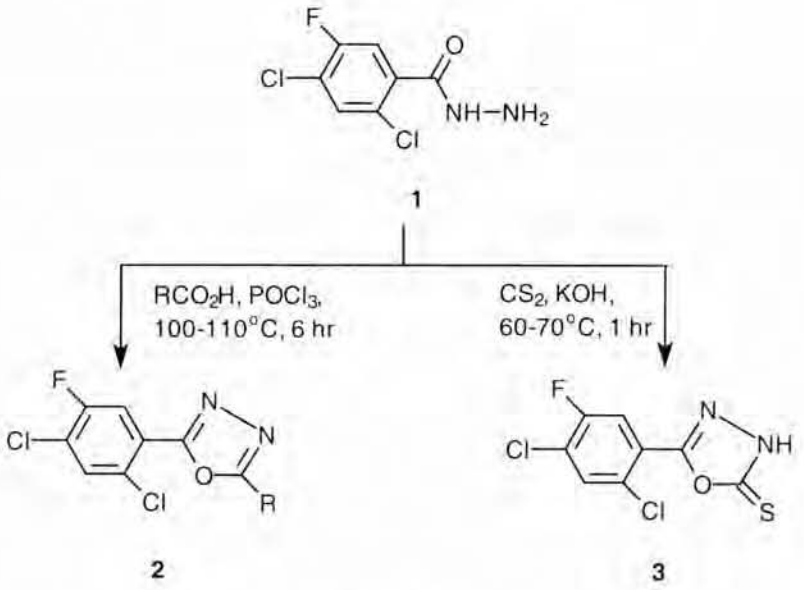
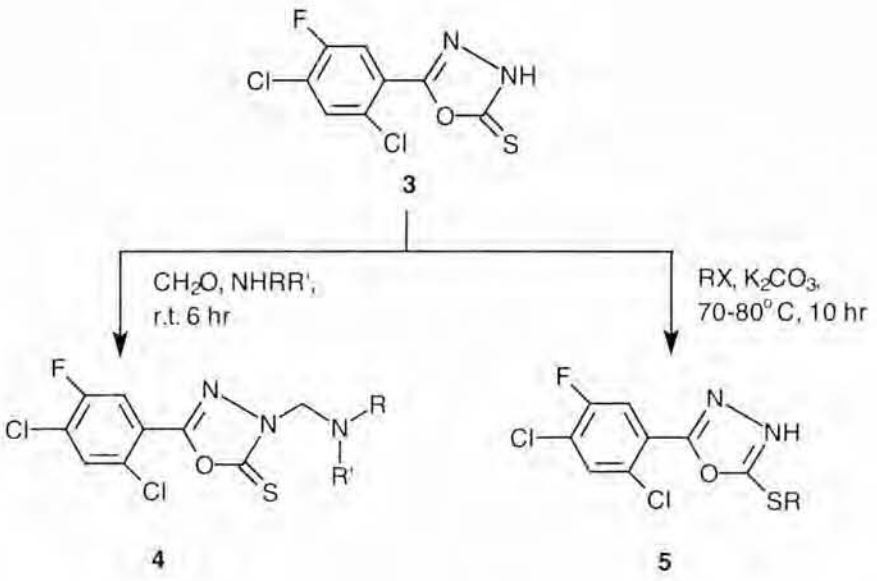


Scheme I



Scheme II

The ^{1}H NMR spectrum of compound **3** a broad peak observed at δ 12.8 was assigned to NH/SH proton. It showed a sharp doublet at δ 7.8 (J=9 Hz) due to H-F ortho coupling. A sharp singlet was also observed at δ 7.4 due to proton at position **3** of 2,4-dichloro-5-fluorophenyl moiety. The mass spectrum of **3** showed stable molecular ion peak at m/z 264 as the base peak. In the IR spectrum of compound **4a** aromatic CH stretch was observed at 3050 cm^{-1}. The CH_2 group of morpholine moiety absorbed at 2956/2857 cm^{-1}. The presence of C=N and C=S bonds were confirmed by the presence of peaks at 1685 and 1571 cm^{-1} respectively. The presence of C-F and C-Cl bonds was also confirmed by their characteristic absorptions at 1120/1018/897 cm^{-1}. The ^{1}H NMR spectrum of compound **4a** is also

Table I — Physicochemical data of 2,5-disubstituted-1,3,4-oxadiazoles **2a-j**

Compd	R	m.p. °C	Mol. formula	Yield (%)
2a	C_6H_5	215	$C_{14}H_7Cl_2FN_2O$	80
2b	4-OMe-C_6H_4-	210-12	$C_{15}H_9Cl_2FN_2O_2$	76
2c	4-Cl-C_6H_4-	228	$C_{14}H_6Cl_3FN_2O$	84
2d	2,4-Cl_2-C_6H_3-	240	$C_{14}H_5Cl_4FN_2O$	72
2e	2,4-Cl_2-5-F-C_6H_2-	>280	$C_{14}H_4Cl_4F_2N_2O$	75
2f	4-Cl-$C_6H_4OCH_2$-	242-44	$C_{15}H_8Cl_3FN_2O_2$	70
2g	2,4-Cl_2-$C_6H_3OCH_2$-	212	$C_{15}H_7Cl_4FN_2O_2$	70
2h	4-F-3-(OC_6H_5)-C_6H_3-	182-86	$C_{20}H_{10}Cl_2F_2N_2O_2$	74
2i	5-(3-Cl-4-F-C_6H_3)-2-furanyl-	116-18	$C_{18}H_7Cl_3F_2N_2O_2$	65
2j	5-(2,4-Cl_2-C_6H_3)-2-furanyl -	155-58	$C_{18}H_7Cl_4FN_2O_2$	65

All the compounds analysed satisfactorily for their N content. They agree to the theoretical values within ± 0.4%. Solvent of crystallization: Ethanol + dioxan mixture.
IR (cm^{-1}) **2b**: 3075(Ar-CH str.), 2842(CH_3 str.), 1690, 1616(C=N str.), 1316/1216(C=O str.), 1118 (C-F str.), 894/923(C-Cl str.). ^{1}H NMR (δ, DMSO-d_6) **2b**: 4.2(s, 3H, -OCH_3), 7.2-7.9(m, 6H, Ar-H). Mass **2b**: 337/338(M^+), 305, 215, 187, 170, 135, 107, 77, 51. IR (cm^{-1}) **2c**: 3100 (Ar-H str.), 1609(C=N str.), 1275(C-O str.), 1189(C-F str.), 1023/908/839(C-Cl str.). ^{1}H NMR (δ, DMSO-d_6) **2c**: 8.4(d, 2H, J= 9.3 Hz, Ar-H), 7.9(d, 1H, $J_{H\text{-}F\ ortho}$=9 Hz), 7.7 (d, $J_{H\text{-}F\ meta}$=3 Hz), 7.5(d, 2H, J= 9.2 Hz, Ar-H). Mass **2c**: 319, 293, 157. Mass **2f**: 372 (M^+), 245, 191, 163, 141, 128, 111, 99, 75, 51. ^{1}H NMR (δ, DMSO-d_6) **2g**: 5.5(s, 2H, -OCH_2), 7.3-7.9(m, 5H, Ar-H). IR (cm^{-1}) **2i**: 3099(Ar-H str.), 1690(C=N str.), 1487(C-N-N str.), 1277/1247(C-O str.), 1106(C-F str.), 961/894(C-F str.). ^{1}H NMR (δ, DMSO-d_6) **2i**: 7.9(d, 1H, $J_{H\text{-}F\ ortho}$= 9 Hz, Ar-H), 7.6(s, 1H, Ar-H), 7.5-6.9(m, 5H, Ar- & furyl H). Mass **2i**: 426/427(M^+), 240, 221, 191, 167, 158, 132, 105, 93, 56.

Table II — Physicochemical data of 2/3, 5-substituted-1,3,4-oxadiazoles (**4a-c** and **5a-c**)

Compd	NRR′/R	m.p. °C	Mol. formula	Yield (%)
4a	Morpholino	146-48	$C_{13}H_{12}Cl_2FN_3O_2S$	70
4b	Methylpiperazino	138-39	$C_{14}H_{15}Cl_2FN_4OS$	65
4c	Piperidino	135	$C_{14}H_{14}Cl_2FN_3OS$	60
5a	CH_3	155	$C_9H_5Cl_2FN_2OS$	90
5b	C_4H_9	128-30	$C_{12}H_{11}Cl_2FN_2OS$	85
5c	C_6H_5CO	170	$C_{15}H_7Cl_2FN_2O_2S$	85

IR (cm^{-1}) **4a**: 3050(Ar-CH str.), 2956/2857(-NCH_2CH_2-O- str.), 1685 and 1571 (-C=N and-C=S str.), 1120/1018/897 (-C-F and -C-Cl str.). ^{1}H NMR (δ, DMSO-d_6) **4a**: 7.9(d, 1H, Ar-H, $J_{H\text{-}F\ ortho}$ =9.2Hz), 7.7(s, 1H, ArH), 7.6(d, 1H, $J_{H\text{-}F\ meta}$ =6.4Hz Mass **4a**: 363, 264, 191, 163, 100. IR (cm^{-1}) **5c:** 3030 (Ar-CH str.), 1690 (C=O str.), 1590 (-C=N str.), 1120/1018/897 (-C-F and -C-Cl str.). ^{1}H NMR (δ, DMSO-d_6) **5c**: 7.2-7.9 (m, 7H, Ar-H). Mass **5c**: 368, 191, 163, 137, 105, 77.

in consistent with its structure. In the IR spectrum of compound **5a** peaks at 3025 and 2985 cm^{-1} was due to the presence of aromatic and aliphatic C-H bonds. Its ^{1}H NMR spectrum showed a doublet at δ 7.6 (J=9 Hz) due H-F ortho coupling. A sharp singlet was also observed at δ 7.3 due to proton at position 3 of 2,4-dichloro-5-fluorophenyl moiety. The protons of CH_3 group resonated as sharp singlet integrating for three protons at δ 4.1. The spectral data for some of the compounds are given under **Table II**.

Biological studies

Antibacterial activity

The newly synthesized compounds were evaluated for *in vitro* antibacterial activity against *Escherichia coli*, *Staphyllococcus aureus* (Smith) and *Klebsiella pneumoniae* (Friedlander) bacterial strains by serial dilution method[18]. Nitrofurazone was used as standard drug for comparison. The results indicate that among tested compounds **2d**, **2e**, **2f**, **3**, **4a**, **4b**, and **5c** are active comparable with that of standard

nitrofurazone (*cf.* **Table III**). The compounds **2e** and **2f** are highly active against all the tested bacterial strains at concentrations comparable with that of standard drug. The activity may be due to the presence of 2,4-dichloro-5-fluorophenyl and 4-chloroaryloxymethyl group. Among Mannich bases **4a** which contains morpholinomethyl unit is the most active one. The activity of **5a-c** are less than or equal to that of its precursor **3**.

Table III — Antibacterial activity data of the compounds (**2a-j**, **4a-c** and **5a-c**)

Compd	Minimum Inhibitory Concentration (μg/mL)		
	E. coli	*S. aureus*	*Klebsiella sps.*
2a	20	20	30
2b	20	20	30
2c	20	20	50
2d	10	10	20
2e	10	10	10
2f	<10	<10	10
2g	<10	<10	25
2h	10	20	30
2i	10	20	20
2j	10	20	30
3	10	10	20
4a	<10	<10	10
4b	10	10	25
4c	10	10	25
5a	20	10	25
5b	20	25	30
5c	10	10	20
Nitrofurazone (Standard)	6	12.5	12.5

Anticancer activity

Nine of the newly synthesized compounds have been screened for their anticancer activity under drug discovery programme of NCI[19-30]. These compounds have been evaluated on 3 cell line one dose primary anticancer assay. The 3-cell lines used in present investigation are NCI-H 460 (Lung), MCF 7 (Breast) and SF 268 (CNS). In this current protocol, each cell line is pre-incubated on microtiter plate, the test agents are then added at a single concentration and the culture incubated for forty eight hours. End point determinations are made with alamar blue. Results for each test agent are reported as the percent growth of the treated cells when compared to the untreated control cells. The compounds which reduce the growth of any one of the lines to 32% or less (negative numbers indicate the cell kill) are considered as active. The compounds **2i** and **4b** are emerged as active in this primary anticancer assay. Compound **2i** and **4b** is active against breast cancer MCF-7 with growth inhibition of 24 and 20 percentages respectively. Results of these studies are given in **Table IV**. These two compounds are passed on for extensive evaluation in the full panel of 60 cell lines over a five- log dose range.

Table IV — Anticancer activity data of 1,3,4-oxadiazole derivatives (**2b-I**, **3** and **4b**).

Compd	Growth percentage (LUNG) (Breast) (CNS) NCI-H 460 MCF-7 SF 268			Activity
2b	98	67	102	Inactive
2c	120	71	84	Inactive
2e	107	116	103	Inactive
2f	69	41	110	Inactive
2g	55	51	77	Inactive
2h	113	114	108	Inactive
2i	38	24	83	Active
3	81	56	110	Inactive
4b	46	20	96	Active

Samples are tested at 1.00×10^{-04} molar concentrations.
Growth percentages less than 32 are considered as active.

Experimental Section

General. Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr pellets on a Perkin-Elmer 157 IR spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on EM 390 300 MHz NMR spectrometer. Mass spectra were recoded in MASPEC low resolution instrument operating at 70 eV. The purity of the compounds were checked by TLC on silica gel plates using petroleum ether:ethyl acetate (3:1) solvent system and iodine was used as visualizing agent.

Synthesis of 2,4-dichloro-5-fluorobenzoic acid. 2,4-Dichloro-5-fluorobenzoic acid was prepared from 2,4-dichloro-5-fluoroacetophenone by haloform reaction. To 2,4-dichloro-5-fluoroacetophenone (0.1 mole) in ethanol was added sodium hydroxide solution until *p*H was 10. Then chlorine gas was passed for several hours (care must be taken to maintain *p*H at 10), until solution smells strongly of chlorine. Then any resinous solid if any was removed. The clear solution so obtained was neutralized with hydrochloric acid to get pure 2,4-dichloro-5-fluorobenzoic acid, yield 90%, m.p. 144-46°C.

Synthesis of 4-fluoro-3-phenoxybenzoic acid. To 4-fluoro-3-phenoxybenzaldehyde (1 eq), hydrogen peroxide (3 eq) and catalytic amount of acetic acid was

added. The mixture was refluxed on at 60-80°C for 2 hr. The solid product separated was filtered, washed with cold water and recrystallized from methanol. The yield of the product is nearly quantitative.

Preparation of ethyl 2,4-dichloro-5-fluorobenzoate. It has been prepared according to the general method of esterification. The compound after usual work-up was obtained as pale yellow oil. It has been judged to be pure by Thin Layer Chromatography (TLC).

Preparation of 2,4-dichloro-5-fluorobenzoyl hydrazine 1. Ethyl 2,4-dichloro-5-fluorobenzoate (0.1 mole), hydrazine hydrate (0.15 mole) and 20 mL of ethanol were refluxed on a water bath for 4 hr. The excess of solvent was distilled off. The compound was recrystallized from ethanol, yield 100%, m.p: 158-62°C.

Synthesis of 2-(2,4-dichloro-5-fluorophenyl)-1,3,4-oxadiazol-5-thione 3. 2,4-Dichloro-5-fluorobenzoic acid hydrazide (0.1 mole), carbon disulphide (0.2 mole) and potassium hydroxide solution (30%, 5 mL) were refluxed on a water bath for 2 hr. The reaction mixture was cooled, acidified and the separated product was purified by recrystallization from ethanol, yield 85%, m.p. 158°C.

Preparation of 2,5-disubstituted-1,3,4-oxadiazole 2a-j. A mixture of aroyl hydrazine (1 mmole), substituted benzoic/aryl furoic acid (1 mmole) and phosphorus oxychloride (2.5 mmole) were refluxed at 100-10°C for 6 hr. Acetonitrile/toluene was used as solvent. The excess of solvent was distilled off and recrystallized from suitable solvent to give oxadiazoles in 65-75% yield (**Table I**).

Synthesis of 3-(methylamino substituted)-5-(2,4-dichloro-5-fluorophenyl)-1,3,4-oxadiazol-2-thione 4a-c. 5-Substituted-1,3,4-oxadiazol-2-thione (1 mmole), formaldehyde (2 mmole) and a secondary amine (1.5 mmole) were taken in ethanol-dioxan mixture and stirred at room temperature for 6hr (refluxed if necessary). The excess of solvent was removed under reduced pressure. The residue was poured into ice cold water and the separated products were crystallized from dioxan. The physicochemical data of the products synthesized are given in **Table II**.

Synthesis of 2-thioalkyl/aroyl-5-(2,4-dichloro-5-fluorophenyl)-1,3,4-oxadiazole 5a-c. 5-Substituted-1,3,4-oxadiazol-2-thione (1 mmole), alkyl/aroyl chloride (1 mmole) and potassium carbonate (5 mmole) in dry ethanol were refluxed for 10 hr. The completion of reaction was checked by thin layer chromatography. The excess of solvent was distilled off and the product was crystallized from suitable solvent. The physicochemical data for the synthesized compounds are given in **Table II**. Their characteristic spectral data are also given in**Table II**.

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References

1 Ghattas A G, El-Sherief H A & Mahmoud, *Pharmazie*, 37, **1982**, 410.
2 Adams S S, Cliffe E E, Lessel B & Nicholson J S, *J Pharm Sci*, 56, **1967**, 1986.
3 Chen B, Qin W, Shen Z & Lei X, *Yiyago Gongye*, 16, **1985**, 305; *Chem Abstr*, 4, **1986**, 186357.
4 Omar A, Mohsen M E & Aboul W O M, *J Heterocycl Chem*, 21, **1984**, 1415.
5 Scot F L, Lambe T M & Butler R N, *J Chem Soc Perkin Trans*, 1, **1972**, 269.
6 Boschelli D H, Connor D T, Kostlan C R, Kramer J B, Mullican M D & Sircar J C, *Eur Pat*, **1991**, 449211; *Chem Abstr*, 116, **1992**, 6570.
7 Gonsalves R, Ph. D. Thesis, Mangalore University, **1999**.
8 El-Ansary S L, Sarhan T Z, Aly E I & Soliman G A, *Bull Fac Pharm*, 31, **1993**, 203.
9 Bettarini F, Caouzzi L, Laporta P, Massimini S & Caprioli V, *Eur Pat*, **1993**, 3276; *Chem Abstr*, 119, **1993**, 49400.
10 Eckhardt W, Berigar E & Zondler H, *Eur Pat*, **1990**, 371925; *Chem Abstr*, 113, **90**, 191385.
11 Pancechowska K D, Foks H, Landowska E, Janowiec M & Zwolska K Z, *Acta Pol Pharm*, 43, **1986**, 116.
12 Yamamoto S, *Jap Pat*, **1993**, 05124948; *Chem Abstr*, 119, **1993**, 146370.
13 Goldstein E J C, *Clin Infect Dis*, 23, **1996**, S25.
14 Niccolai D, Tarsi L & Thomas R T, *Chem Commun*, 24, **1997**, 2333.
15 Filler R & Kobayashi Y, *Biomedicinal Aspects of Fluorine Chemistry*, (Elsevier, Amsterdam), **1982**.
16 Ladva K, Patel P, Upadhyay P & Parekh, *Indian J Chem*, 35B, **1996**, 1062.
17 Smicius R, Jakubkiene V, Burbuliene M M & Vainilavicius P, *Montash Chem*, 133, **2002**, 173.
18 Cruickshank R, Marmion J P & Swain R H A, *Medical Microbiology*, Vol 11, (Churchill Livingstone, London), **1975**, 190.
19 Boyd M R & Paull K D, *Drug Rev Res*, 34, **1995**, 91.
20 Acton E M, Narayanan V L, Risbood P A, Shoemaker R H, Vistica D T & Boyd M R, *J Med Chem*, 37, **1994**, 2185.