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SYNTHESIS OF SUBSTITUTED FLUORO INDOLE DERIVATIVES AS ANTI-INFLAMMATORY AGENTS

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ABSTRACT:
The indole unit is the core structure in a number of natural products. Many indole derivatives are known to exhibit a wide range of biological activity. In the present study, attention has been paid to the synthesis of hetero-cyclic compounds bearing a fluoro indole moiety. 3-Chloro-4-fluoro-benzenediazonium chloride was synthesized in good yield by diazotization of fluoro chloro aniline in presence of sodium nitrite and conc. HCl. 3-Chloro-4-fluoro-benzenediazonium chloride was then treated with 2-Benzyl-3-oxo-butyric acid ethyl ester, sodium acetate and ethanol to give 3-[(3-Chloro-4-fluoro-phenyl)-hydrazono]-4-phenyl-butan-2-one which was then cyclized to get 1-(6-Chloro-5-fluoro-3-phenyl-1H-indol-2-yl)-ethanone **(7).** Five compounds **(7, 9d,9f, 9i and 9j)** were evaluated for anti- inflammatory activity using bovine serum albumin denaturation (*in vitro*) model. Among them **9d** and **9f** demonstrated good anti-inflammatory activity. Here it shows that the synthesized compound possessing electron withdrawing group at *ortho* or *para* position showed better anti-inflammatory activity.

Keywords: Fluoroindole, Anti-inflammatory activity, Bovine serum albumin denaturation, Indole derivatives.

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INTRODUCTION

In the development of organic therapeutic agents, scientists have explored numerous approaches to find and develop organic compounds that are now available to us in dosage forms suitable for the treatment for our illness and often for the maintenance of our health. Heterocyclic are important components of biomolecules such as proteins, DNA, RNA and vitamins and also found in cell lining. Among the heterocyclic compounds, five member heterocyclic moieties fused with aromatic ring systems containing various heteroatoms such as N, S, and O, exhibited wide spectrum of pharmacological activities.

Inflammation is defined as the local response of living mammalian tissue to injury due to any agent. Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin exhibit their anti-inflammatory effect by inhibiting cyclooxygenase (COX) which catalyzes the first step in arachidonic acid metabolism. The chronic use of NSAIDs to treat pain and inflammation is often accompanied by side effects such as gastric ulceration, bleeding, and renal function suppression. It is believed that a selective COX-2 inhibitor will greatly reduce these side effects. Several COX-2 selective inhibitors, including celecoxib (Celebrex), valdecoxib (Bextra), rofecoxib (Vioxx), and etoricoxib (Arroxin), have shown excellent efficacy in humans with few side effects. The modifications of the non-selective NSAID indomethacin have been intensively studied to obtain COX-2 selectivity. It was found that the alkyl, aryl, aralkyl and heterocyclic esters or amides which were modified from indomethacin, exhibit high potency and selectivity[1].

Indole plays an important role as biologically active compound. Indole derivatives constitute an important family of compounds. The compound 5-nitro-2-phenylindole is a promising lead in helping a wide range of antibiotics stay in bacterial cells. Other 2-aryl substituted indole derivatives are implicated in inhibition of bacterial histidine kinase.

Therefore the synthesis and selective fictionalization of indole has been the focus of active research over years. Indole derivatives have been a topic of substantial research interest in heterocyclic chemistry due to their natural occurrence and pharmacological activities. Substituted indoles have been referred to as privileged structures for plenty of pharmacologically active lead compounds in the drug research and development since they are capable of binding to many receptors with high affinity[2]. Indoles are also important synthons for the preparation of biologically active derivatives. On the other hand, it is well accepted that selective introduction of fluorine to organic compounds often causes a marked effect on structure, stability, reactivity, and biological activity. For instance, Celecoxib is a well-known anti-inflammatory agent on behalf of selectively fluorinated indole derivatives. Substituted fluoro indole demonstrated interesting bioactivity. Most of them showed anti-inflammatory[3-7], anticancer[8], antimicrobial[9], antifungal[10].

In the present study, attention has been paid to the synthesis of heterocyclic compounds bearing a fluoro indole moiety. Structural and positional modification was done on the phenyl ring to study their SAR for development of new molecules in this class of anti-inflammatory agents. Some NSAID's like celocoxib and refocoxib are available in the market, which were well known analgesic and anti-inflammatory drugs, but they had certain toxic effects like gastric irritation and agranulocytosis, which made the drugs to be discontinued slowly. In order to overcome the drawbacks of the marketed drugs, it was thought to synthesize some novel indole derivatives, and apply the concept of bioisosterism to get some novel analgesic and anti-inflammatory agents.

MATERIALS AND METHODS

Melting points were determined by Thiel's melting point tube (capillary tube method). The melting points were determined and are uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8300 Shimadzu and the frequencies were expressed in cm-1. 1H NMR spectra were recorded on Bruker-Avance 400 MHz instrument with TMS (0 ppm) as an internal standard. Completion of the reaction and the purity of the compounds were checked on Merck precoated silica gel 60 F-254. Yields were not optimized. Bovine serum albumin (Merck Limited) and other chemicals were of analytical grade. All the solvents and reagents were used without further purification.

Synthesis of 2-Benzyl-3-oxo-butyric acid ethyl ester

70 ml of dry alcohol was taken in 3 naked flask fitted with reflux condenser on magnetic stirrer, 3.85 g of sodium metal added in small portions, 21.6 g (20.97 ml) of ethylacetoacetate was added with stirring during 2 Hrs . Refluxed on steam bath for 4 Hrs & left over night. Ethanol was distilled off. The residue was cooled, diluted with 100 ml of water. Oil separated was extracted with 50 ml of ether. Ether layer was separated and evaporated to get the liquid product. (B. P. 240-242°C).

Preparation of diazonium chloride

The diazonium chloride solution was prepared by adding dropwise 1.4 g (0.02 mole) of sodium nitrite in 20 ml of water to a suspension of (0.02 mole) of substituted aniline in 100 ml of 1N HCl. The reaction mixture was stirred for 1 Hr at 0-5°C and filtered.

Synthesis of 3-[(3-Chloro-4-fluoro-phenyl)-hydrazono]-4-phenyl-butan-2-one

To vigorously stirred solution of 4.4 g (4.4ml) of ethyl – α – benzylacetoacetate in 5 ml of absolute ethanol, add a solution of 0.9 g of NaOH in 2.5 ml water. Immediately after precipitation of gelatinous mass, added 50 ml of water & continued stirring for 4 hr. Unreacted ester was removed by extracting with ether. To the above aqueous layer aryldiazonium chloride solution was added drop wise prepared from appropriate aniline. To the aqueous layer aryl diazonium salt solution was added drop wise and stirred maintaining temperature 0-5°C. After adding 10 g of crystalline sodium acetate stirred for 1hr. The phenyl hydrazone precipitates quickly with the evolution of CO_2. The solid product was filtered, washed with water, aqueous sodium carbonate solution and then again with water and dried. Brown crystals were obtained. Recrystallised it from cyclohexane. Percentage yield 84.30 %, Melting point 220°C, TLC Solvent Ethyl acetate: 1:2, Rf value 0.45.

Synthesis of 1-(6-Chloro-5-fluoro-3-phenyl-1H-indol-2-yl)-ethanone (7)[11]

To the above 3-[(3-Chloro-4-fluoro-phenyl)-hydrazono]-4-phenyl-butan-2- one, dry HCl gas was passed for 2 Hrs. After passing gas the solution was kept aside for 4Hrs at RT and precipitate was obtained. The solution was filtered and dried well. The crude product was recrystallised with ethanol to get the desired product having M.P. 205°C. Percentage yield 57.60 %, Melting point 205°C, TLC Solvent Ethyl acetate: 1:2, Rf value 0.56.

IR spectral data (KBr) cm^{-1}: 3250 cm-1 (NH str secondary amine), 1691 (C=O); 827(Ar- Cl); 1263(C-F).

^{1}H NMR spectral data(δ): 9.21 (s, 1H, Ar-NH), 7.53-7.40 (d, 5H, Ar-H), 7.19-7.15 (d, 2H, Ar-H), 6.99 (d, 1H, Ar-H ortho to fluorine), 2.17 (s, 3H, CH3).

Synthesis of 1-(6-substituted-5-fluoro-3-phenyl-1H-indol-2-yl)-ethanone (9(a-l))

With the above 1-(6-Chloro-5-fluoro-3-phenyl-1H-indol-2-yl)-ethanone (1M), different anilines (3-Nitro aniline, 4-chloro aniline) and phenols (*o*- cresol, *p*-cresol) (1M) were allowed to react separately. 1,4-dioxane 10 ml and 2-3 drops of triethylamine were added and refluxed the reaction mixture 12-14 hr and monitored by TLC. Then it is transferred to ice cold water and filtered the mixture to get the desired products.

1-(5-Fluoro-6-morpholin-4-yl-3- phenyl-1H-indol-2-yl)-ethanone (9a)

IR spectral data (KBr) cm^{-1}: 3250 (NH str secondary amine), 2216(CN), 1691 (C=O), 827(Ar- Cl), 1263(C-F), 1454(CH2),1527(Ar C=C), 2982(CH).

1-(5-Fluoro-3-phenyl-6-piperidin-1-yl- 1H-indol-2-yl)-ethanone (9b)

IR spectral data (KBr) cm^{-1}: 3250 cm-1 (NH str secondary amine), 2216(CN),1691 (C=O), 827(Ar- Cl), 1263(C-F), 1454(CH2),1527(Ar C=C), 2982(CH).

1-(5-Fluoro-3-phenyl-6-phenylamino- 1H-indol-2-yl)-ethanone (9c)

IR spectral data (KBr) cm^{-1}: 3250 cm-1 (NH str secondary amine), 2216(CN), 827(Ar- Cl), 1263(C-F), 1691 (C=O).

1-[5-Fluoro-6-(4-nitro-phenylamino)- 3-phenyl-1H-indol-2-yl]-ethanone (9d)

IR spectral data (KBr) cm^{-1}: 2216(CN), 3250 cm-1 (NH str secondary amine), 1703 (C=O), 1271(C-F), 1444(CH2),1558(Ar C=C), 2985(CH), 1330(C-O), 1228(C-C).

1-[5-Fluoro-6-(3-nitro-phenylamino)- 3-phenyl-1H-indol-2-yl]-ethanone (9e)

IR spectral data (KBr) cm^{-1}: 2216(CN), 1693 (C=O), 1261 (C-F), 3250 cm-1 (NH),1433(CH2), 1523(Ar C=C), 2962(CH), 1261(C-C), 1500(NO2).650 cm-1(C-Cl str).

1-[5-Fluoro-6-(2-nitro-phenylamino)- 3-phenyl-1H-indol-2-yl]-ethanone (9f)

IR spectral data (KBr) cm^{-1}: 2850 cm-1 (C-H str aromatic), 1251 cm-1 (C-C str), 1680 C=O),800 cm-1(C-Cl str), 1500(NO2), 3250 cm-1 (NH).

1-(5-Fluoro-6-phenoxy-3-phenyl-1H-indol-2-yl)-ethanone (9g)

IR spectral data (KBr) cm^{-1}: 2332(CN), 1718 (C=O), 1263(C-F), 1450(CH2) 2854 cm-1 (C-H), 1213cm-1(C-C).

1-(5-Fluoro-3-phenyl-6-p-tolyloxy-1H-indol-2-yl)-ethanone (9h)

IR spectral data (KBr) cm^{-1}: 2332(CN), 1230 (C-C). 1718 (C=O), 3369 cm-1 (NH), 1261(C-F), 1438 (CH2), 1560 (Ar C=C), 2928 (CH), 1103 (C-O).

1-(5-Fluoro-3-phenyl-6-o-tolyloxy-1H-indol-2-yl)-ethanone (9i)

IR spectral data (KBr) cm^{-1}: 2332(CN), 1230 (C-C). 1718 (C=O), 3369 cm-^{1} (NH), 1261 (C-F), 1438 (CH2), 1560 (Ar C=C), 2928 (CH), 1103 (C-O), 1213cm-^{1} (C-C).

1-[5-Fluoro-6-(4-methyl-piperidin-1- yl)-3-phenyl-1H-indol-2-yl]-ethanone (9j)

IR spectral data (KBr) cm^{-1}: 2332 (CN), 1230 (C-C). 1718 (C=O), 3369 cm-^{1} (NH), 1261 (C-F), 1438 (CH2), 1560 (Ar C=C), 2928 (CH), 1103 (C-O).

1-[6-(3-Chloro-4-fluoro-phenylamino)- 5-fluoro-3-phenyl-1H-indol-2-yl]- ethanone (9k)

IR spectral data (KBr) cm^{-1}: 3250 cm-^{1} (NH), 2216 (CN), 1261 (C-F), 827 (Ar- Cl), 1718 (C=O), 2928 (CH).

4-(2-Acetyl-5-fluoro-3-phenyl-1H-indol-6-ylamino)-benzoic acid (9l)

IR spectral data (KBr) cm^{-1}: 3250 cm-^{1} (NH), 827 (Ar-Cl), 1718 (C=O), 2928 (CH), 3390cm-^{1} (-OH), 1560 (Ar C=C), 2928 (CH).

Synthesis of 1-(6-Chloro-5-fluoro-3-phenyl-1H-indol-2-yl)-3-substituted-propenone 11(a-c)

To the above 1-(6-Chloro-5-fluoro-3-phenyl-1H-indol-2-yl)-ethanone derivative (1M), different types of aromatic aldehydes (benzaldehyde, 4-hydroxy benzaldehyde, anisaldehyde were hooked. 8ml of 10 % alcoholic NaOH solution and 25ml solution of main compound was taken in 100ml RBF and stirred it at room temperature for 24Hrs. Then the solvent was poured in ice cold water, filtered, thoroughly washed with water and recrystallised from acetone-water mixture.

1-(6-Chloro-5-fluoro-3-phenyl-1H-indol-2-yl)-3-phenyl-propenone (11a)

IR spectral data (KBr) cm^{-1}: 3250 (NH), 1718 (C=O), 1261(C-F), 1438 (CH2), 1535 (Ar C=C), 2935 (CH), 1103 (C-O), 1230 (C-C).

1-(6-Chloro-5-fluoro-3-phenyl-1H-indol-2-yl)-3-(4-hydroxy-phenyl)- propenone (11b)

IR spectral data (KBr) cm^{-1}: 3250 cm-^{1} (NH), 1718 (C=O), 1261(C-F), 1438 (CH2), 1535 (Ar C=C), 2935 (CH), 1103 (C-O), 1230 (C-C), 3390 (OH).

1-(6-Chloro-5-fluoro-3-phenyl-1H-indol-2-yl)-3-p-tolyl-propenone (11c)

IR spectral data (KBr) cm^{-1}: 3250 cm-^{1} (NH), 1718 (C=O), 1261 (C-F), 1438 (CH_2), 1535 (Ar C=C), 2935 (CH), 1103 (C-O), 1230 (C-C).

***In vitro* anti-inflammatory activity using bovine serum albumin denaturation**

The test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer (0.2M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1ml)

containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27° ±1°C in a water bath for 10 min. After cooling the turbidity was measured at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. The percentage of inhibition is calculated from the following formula[12].

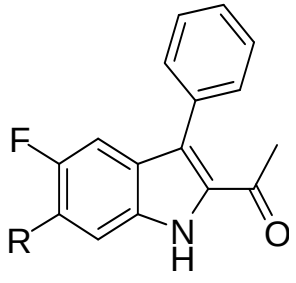
% Inhibition = 100(1-Vt/Vc)

Where Vt = absorbance value in test solution.

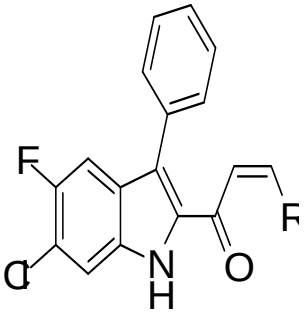
Vc = absorbance value in control solution

DISCUSSION

3-Chloro-4-fluoro-benzenediazonium chloride was synthesized in good yield by diazotization of fluoro chloro aniline in presence of sodium nitrite and conc. HCl. 3-Chloro-4-fluoro-benzenediazonium chloride was then treated with 2-Benzyl-3-oxo-butyric acid ethyl ester, sodium acetate and ethanol to give 3-[(3-Chloro-4-fluoro-phenyl)-hydrazono]-4-phenyl-butan-2-one which was then cyclized to get 1-(6-Chloro-5-fluoro-3-phenyl-1H-indol-2-yl)-ethanone **(7) (Scheme 1).** The keto group has shown in the IR spectra at 1691 cm^{-1}. The resultant compound is treated with five different anilines, phenols and aromatic aldehydes in 1, 4-dioxane, triethyl amine and 10% alcoholic NaOH solution to give different derivatives **(9(a-l)), (11(a-c))**. All the compounds synthesized were identified and characterized by physical methods like Melting point (**Table 1**), Thin layer chromatography and spectral methods like IR and ^{1}HNMR spectra.



9(a-l)



11(a-c)

To investigate the SAR of synthesized fluoro indole derivatives, we have selected the 6th and 2nd position for the preparation of derivatives. Five compounds **(7, 9d, 9f, 9i and 9j)** were evaluated for anti- inflammatory activity using bovine serum albumin denaturation (*in vitro*) model. Among them **9d** and **9f** demonstrated good anti- inflammatory activity given in table 2. Here it shows that the synthesized compound possessing electron withdrawing group at *ortho* or *para* position showed better anti-inflammatory activity.

Table-1: Physicochemical properties of the synthesized compounds 9(a-l), 11(a-c)

Comp.	R	M.P. (°C)	% Yield	R_f value
9a	-morpholin-4-yl	180	63.2%	0.5
9b	piperidin-1-yl	182	59.35%	0.53
9c	-NH-C_6H_5	190	55.83 %	0.58
9d	-NH-C_6H_4-4-NO_2	192	69.23%	0.60
9e	-NH-C_6H_4-3- NO_2	194	68.39%	0.58
9f	-NH-C_6H_4-2- NO_2	196	54.73%	0.61
9g	-O-C_6H_5	184	57.3%	0.58
9h	-O-C_6H_4-4-CH_3	179	37.47%	0.59
9i	-O-C_6H_4-2-CH_3	177	39.46%	0.58
9j	4-methyl-piperidin-1- yl	186	49.32%	0.49
9k	-NH-C_6H_3-3-Cl-4-F	278	45.35%	0.4
9l	-NH-C_6H_4-4-COOH	187	54.65%	0.68
11a	H	260	43.54 %	0.48
11b	4-OH	265	47.76%	0.6
11c	4-CH_3	269	53.63%	0.54

Table 2. In vitro anti-inflammatory activities of compounds (7,9d, 9f, 9i &9j).

S. No.	Comp Code	Anti-inflammatory activity IC_{50} *in μM:*
1	7	300
2	9d	240
3	9f	200
4	9i	>300
5	9j	>300
6	Ibuprofen	125

CONCLUSION

A series of targeted compounds were synthesized and were screened for anti-inflammatory activity *in-vitro* using bovine serum albumin. Among five compounds (7, 9d, 9f, 9i and 9j) compounds 9d and 9f were found to possess good anti-inflammatory activity. In this connection, fluoro indole derivatives bearing electron withdrawing group gives better anti-inflammatory activity. The electron withdrawing groups at *ortho* or *para* position needs to be investigated to develop a class of anti-inflammatory agents.

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