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Synthesis, characterization and evaluation of *In-Vitro* and *In-Vivo* anti inflammatory activity of novel benzimidazole derivatives

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Abstract

The compound 6-chloro-5-fluoro-1H-benzo[d]imidazol-2-amine was treated with various aromatic aldehyde and nickel nitrate using methanol as solvent. These 2-substituted benzimidazole derivatives were synthesized, purified and characterized by means of TLC, Melting point, FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectral analysis respectively. The study aimed at screening of 2-substituted benzimidazole (6a-f) compounds for their *in vitro* and *in vivo* anti-inflammatory activity. All of the synthesized compounds showed good anti inflammatory activity. However the anti inflammatory activity of the synthesized compounds was found to be less than that of respective standard drug at tested dose level.

Key-Words: Benzimidazole, Anti inflammatory, Plethysmograph

Introduction

Inflammation is defined as the local response of living mammalian tissue to injury due to any agent. It is a body defense reaction in order to eliminate or the spread of injurious agent as well as to remove the necrosed cells and tissues[1]. Inflammation that continues unattended can also lead to a host of diseases, such as hay fever, atherosclerosis, rheumatoid arthritis and recent studies include lung cancer. Because of these dangers, the human body closely regulates inflammation in fig 1. NSAIDs work by helping to block the conversion of Arachidonic acid to certain types of PG2s, prostaglandins that orchestrate the process of inflammation in fig 2. When a tissue is injured, prostaglandin synthesis increases in that tissue. The prostaglandins have 2 major actions: They are the mediators of inflammation as well as they sensitize the pain receptors at the nerve endings by lowering the threshold of response to painful stimuli. Moreover allows the other mediators (histamine, bradykinin, 5-HT etc.) which cause inflammation to intensify activation of the sensory neurons. Thus, a drug that prevents synthesis of prostaglandins will be effective in treating pain due to inflammation. The mechanism of action involves the inhibition of cyclooxygenases enzymes in the arachidonic acid cascade for synthesis of prostaglandins [2-4].

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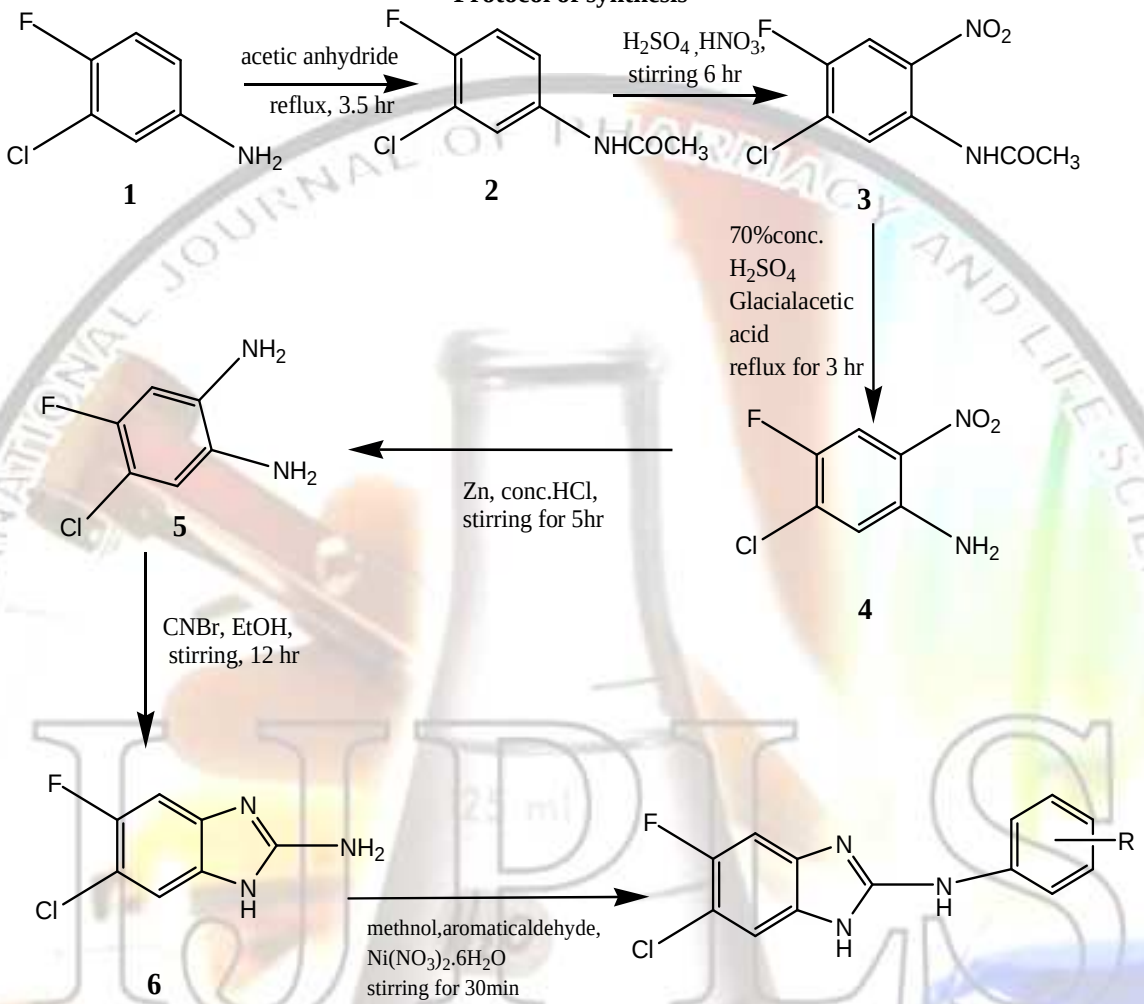
Benzimidazole ring system known to be possessed numerous antimicrobial, anti-inflammatory, anthelmintic, antiviral and anti-tumour properties. Therefore it was enabled that compounds containing benzimidazole nucleus would result in interesting of biological activities [5]. In the same context, our objective of the study was to synthesize such compounds and to further evaluate these synthesized candidates that may exhibit potent anti inflammatory activity [6]. These compounds were also screened for their *in vitro and in vivo* anti inflammatory activity by using diclofenac sodium as standard reference.

Material and Methods

The chemicals used in the present project work were purchased from Rankem, Merck and Spectrochem. The melting point of the synthesized compound was determined by open capillary with Thiel's melting point tube (capillary tube method). TLC plates were prepared by using Merck Silica Gel 60 GF 254. Visualization was done in UV light chamber at 254 nm, iodine chamber. The IR spectra of the synthesized compounds were recorded on a Fourier Transform Infra Red spectrometer (model Shimadzu 8400 S) in the range of 400-4000 cm^{-1} as KBr pellets. (^{1}H NMR) data of the compound was carried out in Bruker 200 spectrospin NMR at Astra Zeneca Pharma India Limited, Bangalore and Bruker 400 spectrospin NMR at Indian Institute of Science, Bangalore. The solvent used for NMR was $CDCl_3$.

Protocol of synthesis



Derivatives

R= 2-OH, N,Ndimethylamino, 4-OH-3-OCH_3, 4-OH, 3,4,5trimethoxy, 2-OH-4-OCH_3

Synthesis of N-(3-chloro-4-fluorophenyl) acetamide (2)

10 g [1 mol] of fluoro chloro aniline and 35 ml of acetic anhydride were placed in a 500 ml RBF. The mixture was refluxed for 3.5 hr, cooled to room temperature and poured in to ice cold water and the volume was reduced to half and then allowed to stand overnight.

Synthesis of N-(5-chloro-4-fluoro-2-nitrophenyl) acetamide (3)

In an RBF was placed 16 ml of sulphuric acid and cooled to 0 ºC in an ice bath. N-(3-chloro-4-fluorophenyl)acetamide5 g (0.02 mol) was added in to RBF with constant stirring followed by slow addition of 11 ml of ice cold concentrated fuming nitric acid over a period of 1 hr. The entire setup was kept in an ice bath for 6 hr with constant stirring. The mixture was slowly poured into a flask containing 1 litre of ice cold water with vigorous stirring until precipitation of solid occurs which was filtered and dried. The solid was dissolved in little acetone then add saturated Na_2CO_3 and then boil it for half an hour cool it and then filter and dry.

Synthesis of 5-chloro-4-fluoro-2-nitrobenzenamine (4)

3 g (0.012 mol) of N-(5-chloro-4-fluoro-2-nitrophenyl) acetamide was dissolved in 15 ml of Glacial acetic acid and 30ml of Con.HCl was added in a 100 ml RBF and

refluxed for 3hr. Then keep it for overnight in room temperature. Orange color crystals appear, decant the upper clear liquid then basify with saturated Na_2CO_3 solution slowly till the effervescence stops.

Synthesis of 5-chloro-4-fluorobenzene-1, 2-diamine (5)

The 5-chloro-4-fluoro-2-nitrobenzeamine (4) 0.5g (0.001) was placed in a RBF along with 10 ml ethanol and 5 g of Zn. To this slow addition of concHCl was done with continuous stirring until all the Zn was consumed. Approximately 40 ml of HCl was required for the addition. The reaction was stirred for 5 hr and poured into ice water. The aqueous layer was basified using saturated NaOH solution till the p^H was 9 and subsequently extracted with ethyl acetate 3 X 20 ml. The organic layer was dried over Na_2SO_4 and solvent removed in vacuum to obtain semisolid mass.

Synthesis of 6-chloro-5-fluoro-1H-benzo[d]imidazol-2-amine (6)

5-chloro-4-fluorobenzene-1, 2-diamine (5) 0.36 g (0.00072 mol) was dissolved in ethanol 10 ml. To the mixture CNBr0.174 g(0.0003 mol) was added in the fuming hood. The reaction was stirred for 12 hr to obtain 6-chloro-5-fluoro-1H-benzo[d]imidazol-2-amine.

Synthesis of Schiff's bases by using different Aromatic aldehydes (6a-f)

The compound 6-chloro-5-fluoro-1H-benzo[d]imidazol-2-amine 0.1g (0.0002 mol) was separately treated with various aromatic aldehyde (1eqivalent) and nickel nitrate (1eqivalent) in methanol stirring for 2hr at room temperature. After 2 hr the solution was poured in to ice cold water. Then extracted with ethyl acetate. The organic layer was dried over Na_2SO_4.

***In vitro and In vivo* screening for anti inflammatory activities[8]**

***In vitro* Anti inflammatory Activity**

A solution of 0.2% w/v of BSA was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 10000 µg/mL of all test each test sample was transferred to 0.1mL Eppendrof tubes using 1mL micropipette. 5mL of 0.2% BSA was added to all the above tubes. The control consists of 5mL 0.2%w/v BSA solution with 50µl methanol. The 0.1mL standard consist 100µg/mL of Diclofenac sodium in methanol with 5mL 0.2%w/v BSA solution. The test tubes were heated at 72^0C for five minutes and then cooled for 10 min. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660 nm. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control.

***In vivo* Anti inflammatory Activity**

The initial paw volume of each rat was noted by mercury displacement method using plethysmograph. Animals in the group-1 was administered with 2.5%DMSO+2.5% tween 20, the group-2 received indomethacin at a dose of 10 mg/kg body weight, where as group 3-10 received the test samples. After the drug treatment, 1% w/v Carrageenan solution (0.1 ml/paw) was injected subcutaneously into the plantar surface of the right hind paw of the rat. The paw volume of the legs of control, standard & tested groups was measured at 4th and 0 h with the help of plethysmograph. Percentage protection (or inhibition) was calculated

By using the formula,

% protection = (1- Vt / Vc) X 100,

Where,

Vt is the mean increase in the paw volume in the test animals group,

Vc is the mean increase in the paw volume in the control group (in anti-inflammatory study).

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The carrageenan test was selected because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation. The subplantar injection of carrageenan in rats leads to paw edema. The early phase (1–2 h) carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissues surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorph nuclear cells and prostaglandins produced by tissues macrophages. The benzimidazole derivatives reduced the carrageenan-induced paw edema in rats. It may be due to inhibition of cyclooxygenase which activates prostaglandin synthesis followed by prevention of inflammatory mediator's release.

Results and Discussion

The starting material flour-chloro-aniline (1) was treated with acetic anhydride to obtain acetyl derivative i.e. fluoro-chloroacetanilide (2) which was confirmed by the change in R_f value and the appearance of C=O peak at 1670 cm^{-1}. Fluoro-chloro acetanilide (2) was nitrated at second position to get flouro-chloronitroacetanilide (3) which was confirmed by the appearance of nitro peak in the IR spectra at 1585 cm^{-1}. The compound obtained is then de acetylated with glacial acetic acid and conc. HCl to get amino compound (4) which was confirmed by the difference in the R_f value and appearance of amino group. The

reduction of compound (4) is done using Zn and HCl to obtain diamine compound (5) which is confirmed by the disappearance of nitro peak around 1500 cm^{-1} and appearance of amino group at 3100-3200 cm^{-1}.The di-amino compound is cyclized to obtain 2-amino benzimidazole compound (6) which is confirmed by the change is R_f value. The ^1H NMR spectra gave peaks at 0.83-0.88 (8H, CH_2 aliphatic); 6.28 (s, 1H, NH_2); 6.52-7.31 (d, 7H, Ar-H); 10.77(d, 1H, NH). These values confirmed the diamine formation and the structure. The 2-aminobenzimidazole compound (6) is then converted into Schiff's bases by treating with nickel nitrate and various aromatic aldehydes leading to the synthesis of 6a-f. Physical and spectroscopical data described in Table 1-3. The compounds 6a-f was screened for anti inflammatory activity using diclofenac sodium as standard reference (100µg/mL) as shown in Table 4. In general, most of the compounds showed significant anti inflammatory activity. However the anti inflammatory activity of the synthesized compounds against the tested organisms was found to be less than that of respective standard drug at tested dose level.

References

1. Mohan GR, Reddy NY, Kumar BV. (2013). Evaluation of analgesic and anti-inflammatory activities of *N*-mannich bases of substituted 2-mercapto-1*H*-benzimidazoles, International Journal of Applied Bio and pharmaceutical Technology, 4 (1):38-46.
2. Gurvinder S, Maninderjit K, Mohan C. (2013). Benzimidazole: The latest information on biological activities, International Research Journal of pharmacy, 4 (1):82-87.
3. Upadhya PK, Uddin K, Sachan K. (2013). Benzimidazole-a medicinally important heterocyclic aromatic organic moiety: an overview, International Journal of pharma world research, 3(2): 1-10
4. Patel P, Pillai J, Darji N, Patel B. (2012). Recent advance in anti inflammatory activity of benzothiazole derivatives, International Journal of Drug Research and Technology, 2 (2):170-176.
5. Sugumaran M, Kumar MY. (2012). Synthesis and biological activity of novel 2, 5disustituted benzimidazole, Indian Journal of pharmaceutical sciences and drug research, 40(1):80-83.
6. Sharma C S, Pandya T, Singh H P, Ankalgi A D, and Ranawat M S.(2012). Synthesis & biological evaluation of some novel mannich bases of benzimidazole derivatives, Indian Journal of Advance Research in Pharmacy and Bio sciences, 1 (1):9-18.
7. Rajasekaran S, Rao GK, Chatterjee A. (2012).Synthesis, anti-Inflammatory and antioxidant activity of some substituted benzimidazole derivatives, International Journal of Drug Developement & Research, 4(3):303-09.
8. Sukhramani PS, Sukhramani PS, Desai SA, Suthar MP. (2011). In-vitro cytotoxicity evaluation of novel Nsubstituted bis-benzimidazole derivatives for anti lung and anti-breast cancer activity, Annual Biochemical Research, 2: 51.
9. Sreena K, Ratheesh R, Rachana M, Poornima M, Shyni C. (2009). Synthesis and anthelmintic activity of benzimidazole derivatives, Hygeia, 1: 21.
10. Mulwad VV, Chaskar AC. (2006). Synthesis and antibacterial activity of new oxadiazolo[1,3,5]- triazine, 1,2,4 triazolo and thiadiazolo 1,3,4 oxadiazole derivatives, Indian Journal of Chemistry, 45B:1710.

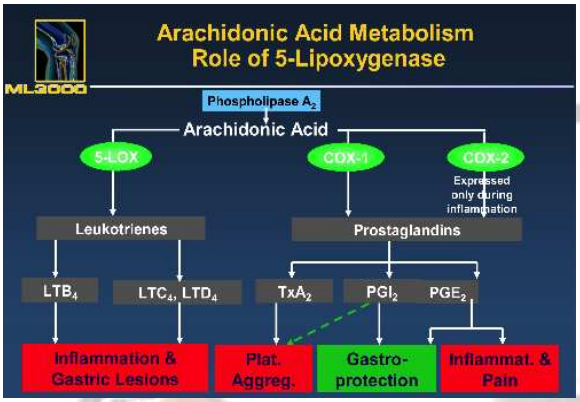


Fig. 1: Flow chart for inflammation

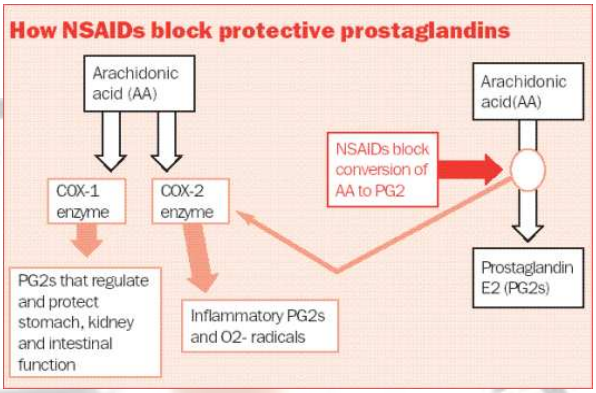


Fig. 2: Mechanism of NSAID'S

Table 1: List of compounds synthesized

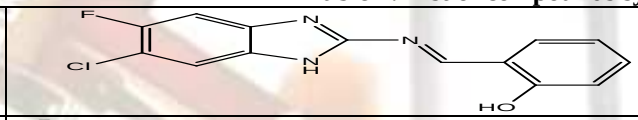
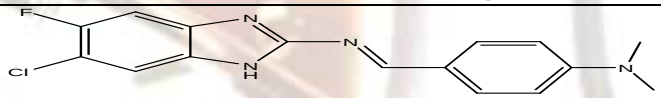
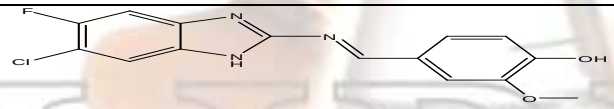
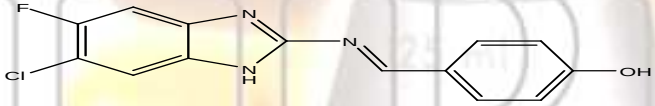
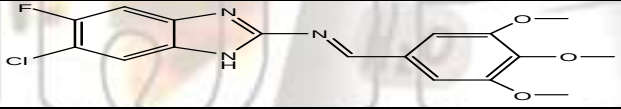
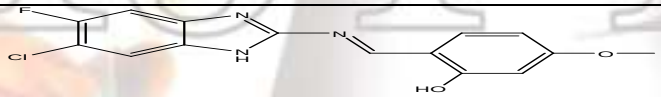
6a		(*E*)-2-((6-chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-ylimino) methyl) phenol.
6b		(*E*)-*N*-(4-(dimethylamino) benzylidene)-6-chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-amine.
6c		(*E*)-4-((6-chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-ylimino) methyl)-2-methoxyphenol.
6d		(*E*)-4-((6-chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-ylimino) methyl) phenol.
6e		(*E*)-*N*-(3, 4, 5-trimethoxybenzylidene)-6-chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-amine.
6f		(*E*)-2-((6-chloro-5-fluoro-1*H*-benzo[*d*]imidazol-2-ylimino) methyl)-5-methoxyphenol.

Table 2: Physicochemical properties of synthesized compounds

Comp Code	Molecular Formula	M.W.	TLC mobile phase	R_f value	Physical state	% yield
6a	$C_{14}H_9FClN_3O$	305	EA: n-Hex 1:1	*0.51*	Semisolid	65%
6b	$C_{16}H_{14}FClN_4$	316	EA: n-Hex 1:1	*0.63*	Semisolid	59%
6c	$C_{15}H_{11}ClFN_3O_2$	319	EA: n-Hex 1:1	*0.52*	Semisolid	85%
6d	$C_{14}H_9ClFN_3O$	289	EA: n-Hex 1:1	*0.62*	Semisolid	70%
6e	$C_{17}H_{15}ClFN_3O3$	363	EA: n-Hex 1:1	*0.50*	Semisolid	75%
6f	$C_{15}H_{11}ClFN_3O_2$	319	EA: n-Hex 1:1	*0.55*	Semisolid	80%

Table 3: Spectral data of the synthesized compounds

Comp. Code	Spectral data
6a	3338 cm^{-1} (NH str secondary amine), 3009 cm^{-1} (C-H str aromatic), 1604 cm^{-1} (C=C str aromatic), 1232cm^{-1}(C-C str), 902 cm^{-1}(C-F str), 3000cm^{-1} (O-H str), 663 cm^{-1}(C-Clstr), 1220cm^{-1} (C-N str)
6b	3350 cm^{-1} (NH str secondary amine), 3009 cm^{-1} (C-H str aromatic), 1604 cm^{-1} (C=C str aromatic), 1232cm^{-1}(C-C str), 908 cm^{-1}(C-F str), 669 cm^{-1}(C-Clstr), 2972cm^{-1} (C-H str aliphatic alkane), 1250cm^{-1} (C-N str)
6c	3350 cm^{-1} (NH str secondary amine), 3009 cm^{-1} (C-H str aromatic), 1604 cm^{-1} (C=C str aromatic), 1220cm^{-1} (C-N str), 1232cm^{-1}(C-C str), 908 cm^{-1}(C-F str), 669 cm^{-1}(C-Clstr), 2931 cm^{-1} (C-H str aliphatic alkane), 1217 cm^{-1} (C-O-C str)
6d	3350 cm^{-1} (NH str secondary amine), 3009 cm^{-1} (C-H str aromatic), 1604 cm^{-1} (C=C str aromatic), 1232cm^{-1}(C-C str), 908 cm^{-1}(C-F str), 669 cm^{-1}(C-Clstr), 1217 cm^{-1} (C-O-C str), 3000cm^{-1} (O-H str), 1250cm^{-1} (C-N str)
6e	3350 cm-1 (NH str secondary amine), 2926 cm-1 (C-H str aromatic), 1604 cm-1 (C=C str aromatic), 1232cm-1(C-C str), 908 cm-1(C-F str), 669 cm-1(C-Clstr), 1217 cm-1 (C-O-C str), 3000cm-1 (O-H str), 2931 cm-1 (C-H str aliphatic alkane), 1220cm-1 (C-N str)
	NMR **In(DMSO-d6) (δ) value in ppm from TMS**
6	6.47-6.53 (s, 2H, NH_2); 7.01-7.182(s, 2H (2 d due to ortho and meta coupling of F); 11.2(s, 1H, NH).
6b	8.08(s, 1H, N=CH); 7.00-7.16(m, 6H, Ar-H); 11.22(s, 1H, NH); 0.88-1.20(m, 6H, CH_3).

Table 4: *In vitro* anti inflammatory activity of synthesized compounds

S/No.	Comp code	IC 50 value in µM
1	6a	27.62
2	6b	7.59
3	6c	8.59
4	6d	17.88
5	6e	32.32
6	6f	26.45
Diclofenac sodium	Std	66.45

Table 5: *In vivo* anti inflammatory activity of synthesized compounds

S/No.	Comp code	%inhibition of paw edema after 4hrs
1	6a	19.26
2	6b	10.74
3	6c	28.16
4	6d	14.07
5	6e	32.02
6	6f	29.76
Diclofenac sodium	Std	61.45