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Der Pharma Chemica, 2011, 3 (5):73-80
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis and biological evaluation of novel pyrazole derivatives as urease inhibitors

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ABSTRACT

*Studies on enzyme inhibition remain an important area of pharmaceutical research since these studies have led to the discoveries of drugs useful in a variety of physiological conditions. The enzyme inhibitors can interact with enzymes and block their activity towards natural substrates. Urease inhibitors have recently attracted much attention as potential new anti-ulcer drugs. A series of novel substituted pyrazoles 8**(a-j)** has been synthesized by diazotization of fluoro chloro aniline (1) and the reaction of the corresponding diazonium salt solution (2**)** with ethyl cyanoacetate **(3)** to give the intermediate, ethyl 2-((3-chloro-4-fluorophenyl) diazenyl-2-cyanoacetate **(4)**. The intermediate is then cyclised with chloroacetonitrile **(5)** using triethyl amine as the base to give the final compound, ethyl 4-amino-1-(3-chloro-4-flurophenyl)-5-cyano-1H-pyrazole-3-carboxylate **(6)**. Nucleophilic substitution group is removed from the final compound and **8(a-j)** derivatives have been synthesized. All the synthesized compounds were characterized by physical data (M.P. & TLC) and spectral Data (IR & ^{1}H NMR). The synthesized compounds were evaluated for urease-inhibition activity. Molecular docking studies were carried out for these compounds with the enzyme urease. From the observations it has been noticed that some of the compounds possesses remarkable urease-inhibitory effect.*

Key words: fluoropyrazole, urease inhibition activity, Nessler's Reagent, docking, *insilico.*

INTRODUCTION

Urease is directly involved in the formation of infection stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma

and urinary catheter encrustation.urease is known to be the major cause of pathologies induced by helicobacter pylori (HP) which allow HP to survive at low pH of the stomach and therefore play an important role in pathogenesis of gastric and peptic ulcer which in some cases may progress of cancer [1-3].

Literature survey reveals that the Urease inhibition having some medically importance against to treat ulcer [4-8]. In recent year heterocyclic compounds analogues and derivatives have attracted strong interest due to their useful biological and pharmacological properties.

Pyrazole and its derivatives are known to be biologically active compounds. And Substituted pyrazoles have shown wide range of biological activities like antioxidant[9,16] antibacterial[9,11,13,16],antifungal[14,23],analgesic[15],lipidperoxidation[15],antiinflammatory[9,12,15], Anti atherosclerosis [17], antidepresent [18], antitumour activities [21], etc .In view of these and our continuing interest in the synthesis of biologically active compounds. Hence we have chosen the title topic for our research.

The most recent phase in the new drug discovery process is utilizing the knowledge of the three dimensional structures of target macromolecules or of related proteins. The same strategy is used in present study to know the binding of the newly synthesized pyrazoles to selected protein sites. It was theoretically predicted that how inhibitors bind to the molecular targets, specific interactions that are important in the molecular recognition

MATERIALS AND METHODS

Standard chemicals are purchased from chemfort (India) Pvt.ltd. And sd fine Chemical Pvt ltd. Melting points were determined in open capillaries and are uncorrected.IR spectra were recorded Shimadzu FTIR5400. Infrared spectrophotometer using KBr.1HNMR spectra were recorded on Perkin-Elmer, 90 MHz spectrophotometer. Using TMS as standard (chemical shift in δ ppm)

General procedure for Synthesis of 1-chloro-2-(3-chloro-4-fluorophenyl) diazene (2):
A mixture of (0.18 mol) of fluoro chloro aniline with 46ml of conc. HCl and 75 ml of water taken in a glass mortar. Transferred this suspension to a 500 ml RBF and stirred well. Cooled the contents of the flask in an ice bath to 0-5°C and, added a solution of 13.0g (0.19 mol) of sodium nitrite in 175 ml of water from a dropping funnel during about 20 minutes. Kept the diazonium salt solution below 5°C and, filtered.

Synthesis of ethyl 2-((3-chloro-4-flurophenyl) diazenyl)-2-cyanoacetate (4):
Sodium acetate (20 mmol) was added to ethyl cyanoacetate (17.3 mmol), which was dissolved in ethanol (120 ml), cooled below 5°C, and the previously prepared diazonium salt was added with vigorous mechanical stirring. Stirring was continued for 2 hr at room temperature. The precipitate was filtered off, washed twice with water and finally dried at 50°C the synthesized compounds were characterized by elemental and spectral analysis.

FT-IR cm^{-1}spectra of Comp No. 4
Data: 2222(CN); 2978.19(CH), 1465 (CH_2) 1718 (C=O); 761(Ar- Cl); 1263(C-F).

^{1}H NMR spectra of Comp No. 4 Data: 7.502-7.474 (d, 1H, J=5.45, CH at I), 7.2097.131(d, 2H, J=6.55, CH at II and III). 1.634(s, 1H, CH at IV).4.431, (q, 2H, CH_2 at V).1.370 (t, 3H, CH_3 at VI)

Synthesis of substituted ethyl 4-amino-1-(3-chloro-4-fluorophenyl)-5-cyano-1H-pyrazole-3-carboxylate(6):
To the intermediate (4) 1mmol chloroacetonitrile (1ml) was added and then NEt_3 (5 mmol) drop wise with external cooling. Mixture was heated at 80-90°C during 1-4hours. After cooling, water was added (10-15ml) and brown oil separated. Water was decanted and the oil dissolved in ethyl acetate, dried and evaporated to produce brown oil again.

The oil was scratched with 10 percent solution of ethyl acetate in ether, solidified and the product was filtered and washed with ether, which yielded a brown solid.

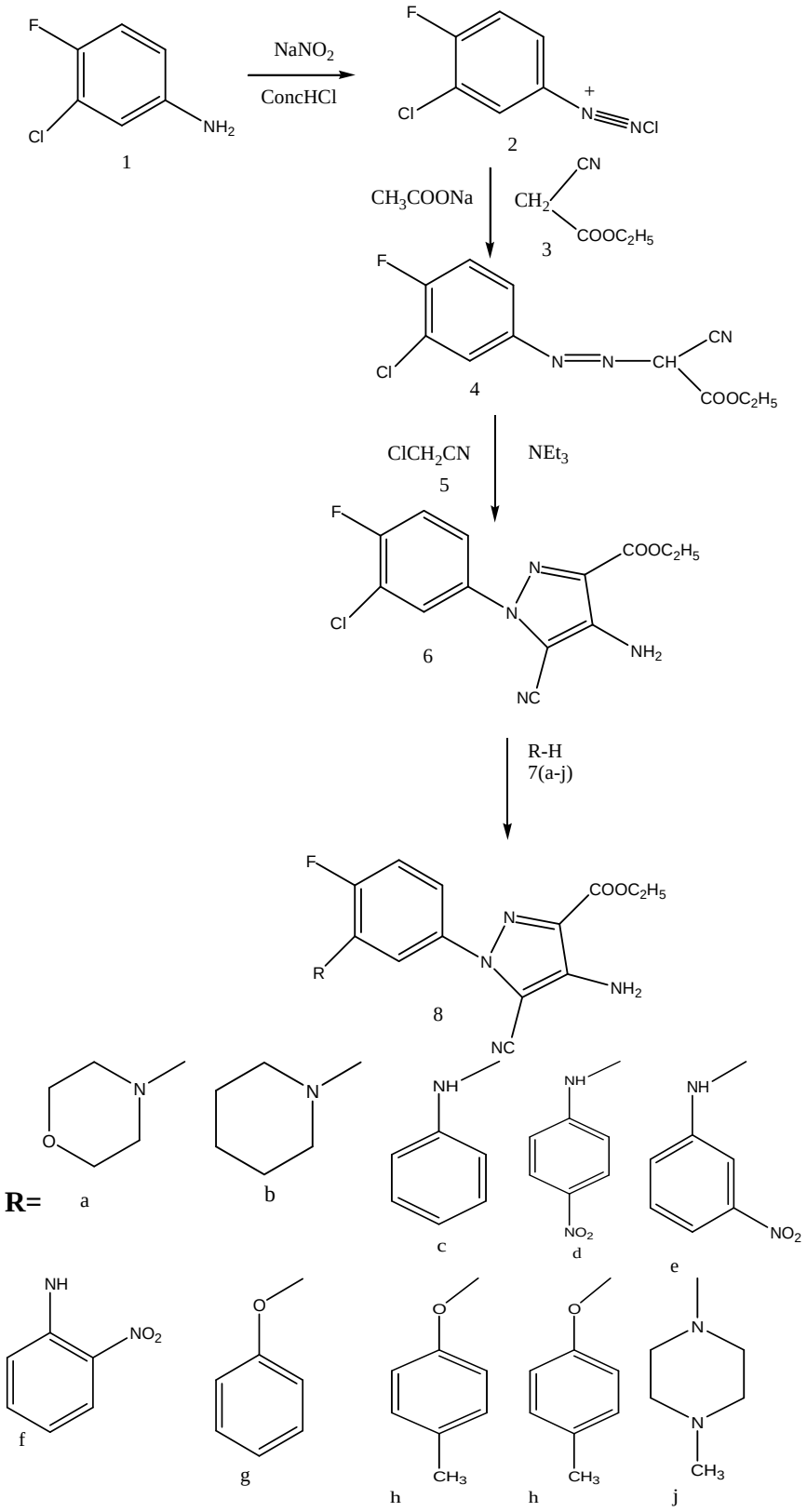
FT-IR cm^{-1} spectra of Comp No. 6 Data: 3390(NH_2); 2216(CN) 1691 (C=O); 827(Ar- Cl); 1263(C-F); 1454(CH_2)1527(Ar C=C); 2982(CH).
^{1}H NMR spectra of Comp No. 6 Data**:** 7.822-7.778 (m, 1H, CH at I). 7.359-7.317 (d, 1H, CH at II). 7.644-7.567 (m, 1H, CH at III). 4.527-4.414 (m, 2H, NH_2 at IV). 1.589 (m, 2H, CH_2 at V). 1.475-1.404(t, 3H, CH_2 at VI)

Synthesis of substituted ethyl 4-amino-1-(3-substituted-4-fluorophenyl)-5-cyano-1H-pyrazole-3-carboxylate (8a-j):
To the above ethyl 4-amino-1-(3-chloro-4-fluorophenyl)-5-cyano-1H-pyrazole-3-carboxylate derivative (1mol), different types anilines(3-Nitro aniline, 4-chloro aniline) or phenol (*o*- cresol, *p*- cresol) (1mol), 1,4-dioxane 10 ml and 2-3 drops of triethylamine was taken in a 100 ml RBF fitted with a reflux condenser. Refluxed the reaction mixture 12-14 hr and monitored by TLC, then transferred to ice cold water and filtered the solution to get the desired products.

FT-IR cm^{-1} spectra of Comp No. 8a
Data: 3232(NH_2); 2216(CN); 1703 (C=O); 1271(C-F); 1444(CH_2); 1558(Ar C=C); 2985(CH); 1330(C-O), 1228(C-C)**.**
FT-IR spectra of Comp No. 8i Data: 3367(NH_2); 2360(CN); 1718 (C=O); 1261(C-F); 1438(CH_2); 1535(Ar C=C); 2935(CH); 1103(C-O), 1230(C-C).

Biological Evaluation
Urease inhibition activity
Enzyme Source. Fresh Watermelon was purchased from main vegetable markets in Bangalore in February 2011. And authenticated by Botanist,

Preparation of Extract
5gm of Watermelon seeds are soaked in water for overnight. Homogenized the soaked seeds with 50ml of 100mM phosphate buffer pH 6.8 and allowed to stand for 30mins with occasional agitation. The suspension was then centrifuged at 3000rpm for 15min.Decant the supernatant into a clean glass beaker, which contains urease and stored in cold condition.


NaNO2
ConcHCl
CH3COONa
ClCH2CN
NEt3
R-H
7(a-j)
R=
a
b
c
d
e
f
g
h
h
j

Standard curve for ammonia estimation

0.2-1.0 ml different aliquots of standard ammonium sulphate solution and water taken in to different test tubes followed by 1.0ml of Nessler's reagent to all the test tubes. Read the absorbance at 490nm against blank. (Table 1)

Enzyme assay

In Two different test tubes blank and test. Taken 1.0 ml of 100Mm of phosphate buffer, 1.0ml of urea solution and. 0. 2 ml Enzyme extract is added to the test only and incubate at37°c in a water bath exactly for 30minutes, with constant gentle shaking. 7N Sulphuric acid is added to each test tube to arrest the reaction. 0.2ml of enzyme extract is added to the blank.

After completion of enzymatic reaction both in blank and test, centrifuge the contents at room temperature for 15mins at 3500rpm. 0.2 ml of the supernatant is used for ammonium Estimation. (Table 2)

Molecular Docking Studies:

The newly synthesized compounds were subjected for molecular docking by calculating the minimum energy by inhibiting the target protein involved in the inflammation pathway. The ligands were drawn in ChemDraw Ultra 6.0 assigned with proper 2D orientation (Chem Office package) and the structure of each ligand was analyzed by using Chem-3D Ultra 6.0 (ChemOffice package) and was checked for the connection error in bond order. ADMET property was achieved through Pre ADMET server- a web-based application for predicting ADMET data and building drug-like library using *in silico* method. Energy of the molecules was minimized using Dundee PRODRG2 Server. Then the file was opened in SPDB viewer and C-terminal Oxygen was added using fit module property. CASTp (Computed Atlas of Surface Topography of proteins) server was used to identify the active pockets on target protein molecules. Autodock V3.0 was used to perform Molecular Docking. (Table 4)

Table 1. Standard curve for ammonia estimation

Sl.No	Vol of ammonium sulphate sol (ml)	Conc. Of ammonia(µg)	Vol of distill water (ml)	Nessler's reagent(ml)	0.D at 480nm
1	0.0	0.0	12.0	1.0	0.00
2	0.2	2.0	11.8	1.0	0.02
3	0.4	4.0	11.6	1.0	0.04
4	0.6	6.0	11.4	1.0	0.06
5	0.8	8.0	11.2	1.0	0.08
6	1.0	10.0	11.0	1.0	0.10

Table 2. Enzyme Assay

	Po_4 Buffer	Urea	H_2So_4	Enz	Nessler's	Water	O.D	Activity (µmol/ml/min)
Ec(Control)	--	1ml	0.5 ml	0.2 ml	1.0ml	10.3ml	0.0	----
Et(Test)	0.2 ml	1ml	0.5 ml		1.0ml	10.3ml	0.55	0.12

Table 3.Inhibition studies

Compound No	Vol of enzyme + inhibitor	Vol of distill water	Nessler's reagent (ml)	O.D at 480 nm	Activity (µmol/ml/min)
4	0.1+0.1	11.8	1.0	0.09	0.0247
6	0.1+0.1	11.8	1.0	0.33	0.06594
8b	0.1+0.1	11.8	1.0	0.41	0.08242
8c	0.1+0.1	11.8	1.0	0.68	0.1569
8d	0.1+0.1	11.8	1.0	0.64	0.1348

Table 4: Molecular docking details of all the compounds with Urease enzyme

Molecule	**Binding energy**	**Docking energy**	**Inhibitory constant**	**Intermol energy**	**H-bonds**	**Bonding**
4	-3.25	-1.91	0.0	-4.81	2	UR:A:MET637:HN::4:DRG1:OAO UR:A:GLY638:HN::4:DRG1:O
6	-6.03	-6.49	3.82e-005	-6.34	2	UR:A:MET637:HN::6:DRG1:NAO UR:A:GLY638:HN::6:DRG1:NAO
8a	16.01	15.07	--	14.45	--	----------
8b	15.04	14.42	--	13.48	1	UR:A:ARG639:HH11::8b:DRG1:OAX
8c	21.2	20.51	--	19.33	--	----------
8d	34.61	34.47	--	32.43	--	----------
8e	27.03	28.17	--	24.85	--	----------
8f	26.07	30.02	--	23.89	--	----------
8g	45.19	44.85	--	43.32	--	----------
8h	48.20	40.16	--	42.09	--	----------
8i	50.03	51.3	--	46.70	--	----------
8j	52.34	55.67	--	58.71	--	----------

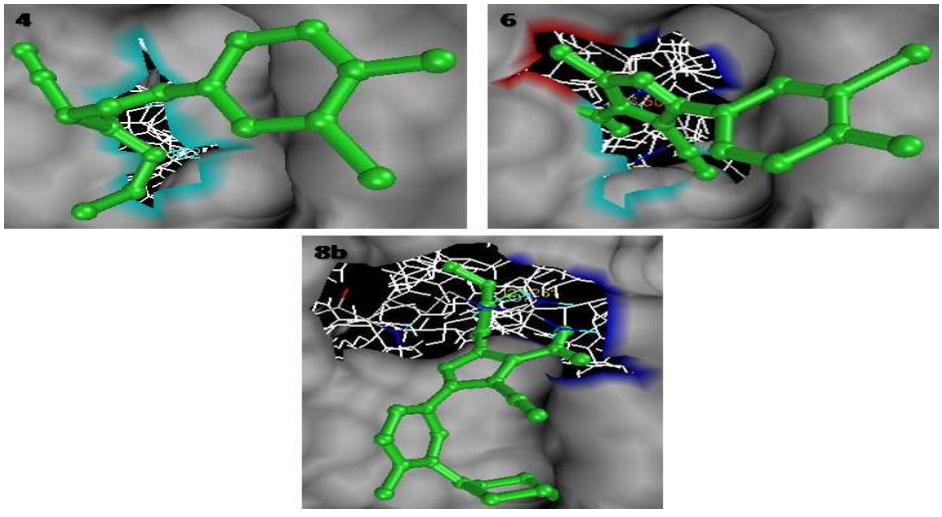


Fig 1: Docking postures of the compounds 4, 6 and 8b with Urease showing hydrogen bonds.

RESULT AND DISCUSSION

Urease was extracted from watermelon seeds and screened its inhibition studies using substituted fluoro pyrazole derivatives. We found that. The synthesized compounds No 4, 6, and 8b have very good inhibition activity against urease.

The docking postures are shown in fig 1. Binding energy and hydrogen bonds formed are tabulated in the table. The theoretical outcome highlight that the minimum binding energy of the molecules with the targeted protein may make these newly synthesized pyrazoles as good inhibitors for inflammation. However, these promising results are reliable and further can be subjected for preclinical studies to arrive at the conclusion on these molecules for their clinical use.

Acknowledgement
Authors are thankful to Nargund College of pharmacy for providing laboratory facilities. To Carry out this research work.

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