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Synthesis and biological evaluation of 6-fluoro benzothiazole substituted pyrazolo azetidinones

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ABSTRACT

In the present work, 2-amino benzothiazoles were synthesized from 3-chloro-4-fluoro aniline and further condensed with 3-methyl-1-phenyl-5-Pyrazolone to yield the corresponding Schiff's base. The Schiff's base was cyclised with chloroacetyl choride in triethylamine to yield 2-azetidinones. The formed 2-azetidinones were further condensed with different primary and secondary amines. The synthesized compounds were confirmed by spectral analysis such as IR, ^{1}H-NMR, Mass and were screened for Anti-inflammatory, Anti-diabetic, Anti-oxidant and Anti-microbial activity.

Key Words: 2-Aminobenzothiazole, Azetidinone, Anti-inflammatory, Anti-diabetic, Antioxidant

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INTRODUCTION

The chemistry and biological study of heterocyclic compounds has been an interesting field in medicinal chemistry for a long time. 2-amino benzothiazole, a heterocyclic compound containing N and S atoms serve as a unique and versatile scaffold for experimental drug design[1] and have varied biological activities like anti-inflammatory, anti-tumour[2], anthelmintic[3], anti-tubercular[4], anti-convulsant[5] and antimicrobial[6]. Pyrazoles and Pyrazolones are the key structures in numerous compounds. They represent an important class of compounds not only for their theoretical interest but also for their biological activities such as anti-inflammatory[7], hypoglycemic[8] and antimicrobial[9]. The presence of Azetidinone or β-lactam ring in several families of bicyclic antibiotics had stimulated continuous interest because of their high antibacterial activity[10]. Azetidinones can be prepared from Schiff's bases which are the condensation products of carbonyl and amino compounds. They are considered to be significant owing to their wide range of biological applications like antiparkinsonism[11], cholesterol absorption inhibitors[12], anticonvulsant[13] and antimicrobial[14]. In present study, a novel series of azetidinone derivatives were synthesized from pyrazolo-benzothiazoles and were screened for antidiabetic, anti-inflammatory, antioxidant and antimicrobial activities.

MATERIALS AND METHODS

The synthesized compounds are first purified by recyrstallisation usind appropriate solvents. The melting points were determined in an open capillary tube and are uncorrected. The IR spectra were recorded on ABB BOMEM FTIR Spectrometer using KBr disc of the sample. The NMR spectra were recorded as 400MHz NMR Spectrometer in DMSO using TMS as an internal standard. Chemical shift is given in δppm.

Synthesis of 2-amino-6-fluoro-7-chloro (1,3) benzothiazole
To glacial acetic acid (20ml) cooled below room temperature were added 8gm (0.08mol) of potassium thiocyanate and 1.45g (0.01 mol) of fluoro chloro aniline(Compound 1). The mixture was placed in a water bath and stirred with

magnetic stirrer while 1.6ml of bromine in 6ml of glacial acetic acid was added from a dropping funnel at such a rate that the temperature never rises beyond room temperature. After all the bromine was added (105min), the solution was stirred for 2 hours below room temperature and at room temperature for 10 hours, it was then allowed to stand overnight, during which period an orange precipitate settle at the bottom, water (6ml) was added quickly and slurry was heated at 85^0C and filtered hot. The orange residue was placed in a reaction flask and treated with 10ml of glacial acetic acid heated again to 85^0C and filtered hot. The combined filtrate was cooled and neutralised with ammonia solution to the pH range 6.0 A dark yellow precipitate was collected. Recrystalised from benzene, ethanol of (1:1) after treatment with animal charcoal gave yellow crystals of 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole.

Scheme

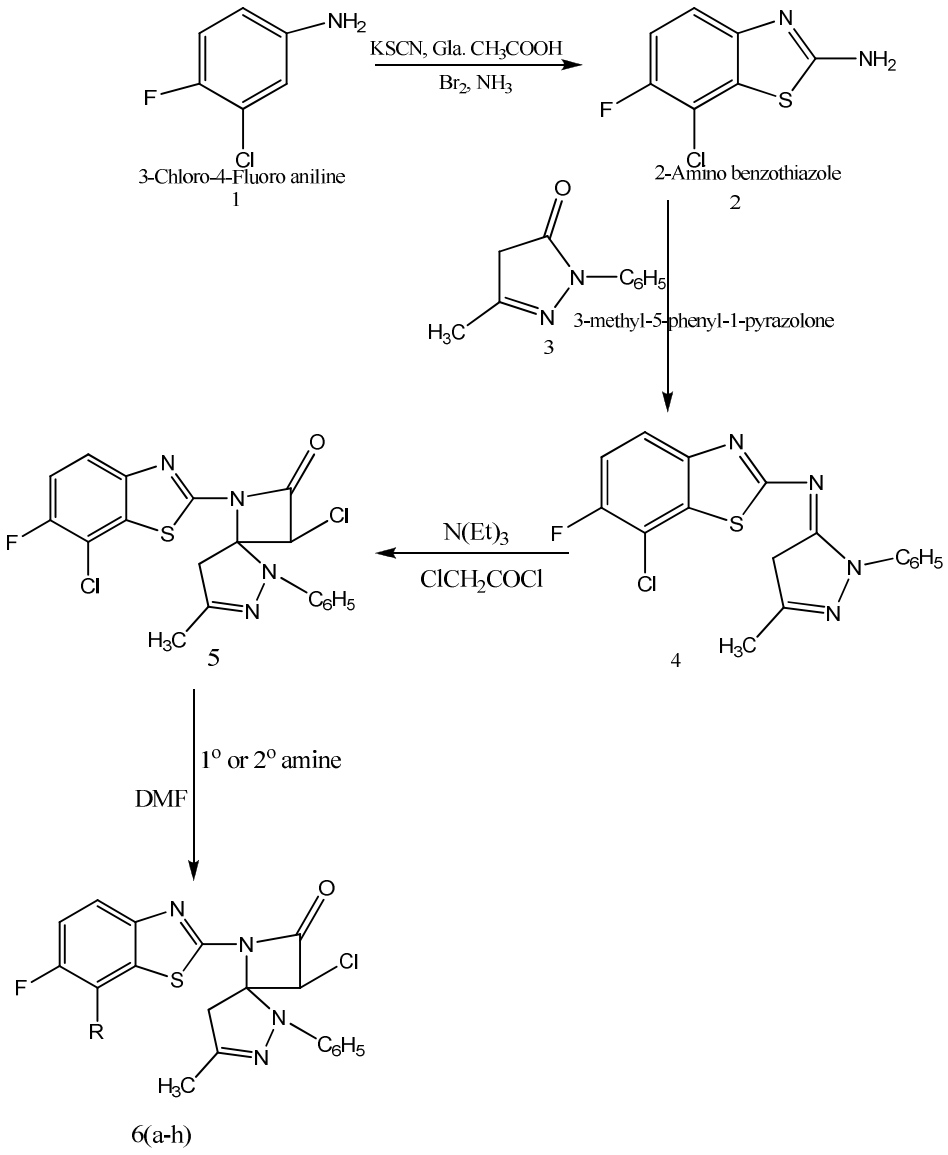

Table no:1 Details of the synthesized compounds

Compound code	Amine
6a	o-Anisidine
6b	m-Anisidine
6c	p-Anisidine
6d	o-Toluidine
6e	m-Toluidine
6f	p-Toluidine
6g	β-phenylethylamine
6h	Morpholine

Synthesis of 3-methyl-1-phenyl-5-Pyrazolone
In 250 ml R.B flask, to 6.7ml of phenyl hydrazine hydrochloride, 9.3 gm of sodium acetate crystal, 6.5ml of ethylacetoacetate, add 10 ml of ethanol and reflux the mixture for 1 hour on water bath and kept aside for overnight. The separated product was recrystallised from toulene and wash with water to get 3-methyl-1-phenyl-5-Pyrazolone.

Synthesis of Schiff's base
A mixture of Compound 2(0.01mol) and Compound 3(0.01mol) was dissolved in absolute ethanol. The mixture was refluxed for 6hrs. It was then concentrated to remove excess of alcohol and cooled and poured into ice in small portions. The resulting separated solid was filtered off, dried and recrystallised with benzene and ethanol.

Synthesis of Substituted Azetidinones
To a cold mixture of chloroacetyl chloride(0.012mol) and triethylamine(0.02mol), a well stirred mixture of Compound 4(0.0.1mol) and 1,4-dioxane(50ml) was added dropwise at 0°C.

The reaction mixture was then stirred for 18 - 20 hrs and kept aside for 3 days at room temperature. The product obtained was then recrystallised from ethanol to yield Substituted Azetidinones.

Synthesis of 3-chloro-1-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl0-7-methyl-5-phenyl-1,5-triazaspiro[3,4]oct-6-en-2-one
To the Compound 5(0.01mol) dissolved in DMF, primary or secondary amine(0.01mol)added and refluxed for 2hrs. The mixture was cooled and poured into crushed ice. The separated solid was filtered, dried and recrystallised.
TLC; mobile phase: n-butanol: ethylacetate: benzene-1:4:1

Table no: 2 Physical characterizations of the synthesized compounds

Compound	MOL. FORM	Mol.Wt	Rf value	M.P °C	% Yield	Elemental analysis (Calculated)		
						C	H	N
6a	$C_{26}H_{21}O_2SN_5FCl$	521	0.65	107	65%	59.82	4.05	13.42
6b	$C_{26}H_{21}O_2SN_5FCl$	521	0.64	108	67%	59.82	4.05	13.42
6c	$C_{26}H_{21}O_2SN_5FCl$	521	0.67	110	70%	59.82	4.05	13.42
6d	$C_{26}H_{21}OSN_5FCl$	505	0.71	160	68%	61.72	4.18	13.84
6e	$C_{26}H_{21}OSN_5FCl$	505	0.71	159	59%	61.72	4.18	13.84
6f	$C_{26}H_{21}OSN_5FCl$	505	0.73	162	69%	61.72	4.18	13.84
6g	$C_{27}H_{23}OSN_5FCl$	485	0.72	103	71%	62.36	4.46	13.47
6h	$C_{23}H_{21}O_2SN_5FCl$	520	0.69	96	69%	56.85	4.36	14.41

Spectral analysis:
Compound 6a:
IR (KBr, cm^{-1}): 3012.56(Ar-CH), 1356.3(Ar-NH), 1595(C=N), 755(C-Cl), 1650(ArC=O), 1160(C-S). ^{1}H-NMR (DMSO, δ in ppm): 6.7-7.5(m, Ar-H, 11H), 2.11(s, C-CH_3, 3H), 4.1(s, N-H, 1H), 3.06(d, CH-H_A, 1H), 3.31(d, CH-H_B, 1H), 5.26(s, CH-Cl, 1H), 3.83(s, O-CH_3, 3H).

Compound 6d:
IR (KBr, cm^{-1}): 3011.52(Ar-CH), 1336.21(Ar-NH), 1644(C=N), 744(C-Cl), 1707(ArC=O), 1191(C-S). ^{1}H-NMR (DMSO, δ in ppm): 6.5-7.4(m, Ar-H, 11H), 2.11(s, C-CH_3, 3H), 4.3(s, N-H, 1H), 3.06(d, CH-H_A, 1H), 3.31(d, CH-H_B, 1H), 5.28(s, CH-Cl, 1H), 2.12(s, C-CH_3, 3H).

Compound 6g:
IR (KBr, cm^{-1}): 3041.56(Ar-CH), 1229.11(Ar-NH), 1604(C=N), 749(C-Cl), 1643(ArC=O), 1190(C-S). ^{1}H-NMR (DMSO, δ in ppm): 6.7-7.4(m, Ar-H, 11H), 2.11(s, C-CH_3, 3H), 3.8(s, N-H, 1H), 3.06(d, CH-H_A, 1H), 3.31(d, CH-H_B, 1H), 5.28(s, CH-Cl, 1H), 3.4(t, Ar-N-CH_2, 2H), 2.93(t, N-CH_2-C, 2H).

Compound 6h:
IR (KBr, cm^{-1}): 3039.42(Ar-CH), 1306.11(Ar-NH), 1630(C=N), 753(C-Cl), 1690(ArC=O), 1196(C-S). ^{1}H-NMR (DMSO, δ in ppm): 6.7-7.2(m, Ar-H, 11H), 2.11(s, C-CH_3, 3H), 3.06(d, CH-H_A, 1H), 3.31(d, CH-H_B, 1H), 5.28(s, CH-Cl, 1H), 3.18(t, N-$(CH_2)_2$, 4H), 3.65(t, CH_2-O-CH_2, 4H).

***In-vitro* Anti-diabetic Screening[15]:**
The synthesized compounds were subjected to *in-vitro* anti-diabetic screening by using α-amylase inhibition activity based on colorimetric method and Acarbose was used as control for comparision. A starch solution (0.1% w/v) was obtained by stirring 0.1g of potato starch in 100ml of 16 mM of sodium acetate buffer. The enzyme solution was

prepared by mixing 27.5mg of α-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 96mM 3,5 di-nitro salicylic acid solution. Both control (Acarbose) and derivatives were added with starch solution and left to react with α-amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3,5-dinitro salicylic acid to 3-amino-5- nitro salicylic acid. This reaction is detectable at 540 nm. (Temperature 25ºC±0.1 ºC, pH 4.8; O.D. at 540 nm). The results were tabulated in Table.3

Table no.3 Anti-diabetic sreening of the synthesized compounds

Compound	% Alpha amylase inhibition					IC_{50} values (µg/ml)
	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	
6a	23.19	36.98	50.21	60.42	75.15	200
6b	18.42	34.64	52.93	69.62	80.65	190
6b	32.2	40.29	53.35	67.96	76.68	180
6d	21.39	30.25	42.39	51.05	63.43	260
6e	16.5	27.22	34.0	43.56	55.49	330
6f	45.52	55.04	75.0	87.28	96.5	120
6g	31.5	46.9	59.97	68.50	73.31	130
6h	28.53	40.06	57.53	71.70	84.34	160
Acarbose	43.63	55.5	74.2	88.13	96.8	63

$$\% \text{ inhibition} = \frac{[Abs_{540\ (Control)} - Abs_{540\ (Test)}] * 100}{[Abs_{540\ (Control)}]}$$

***In-vitro* Anti-inflammatory Screening[16]:**

The synthesized compounds were screened for *in-vitro* anti-inflammatory activity using Protein denaturation method. The standard drug and test compounds were dissolved in minimum amount of dimethyl sulfoxide (DMSO) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMSO in all solutions was less than 2.0%. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1% mM Bovine albumin solution in phosphate buffer and incubated at $27^0 \pm 1^0$C in incubator for 15 min. Denaturation was induced by keeping the reaction mixture at $60^0 \pm 1^0$C in water bath for 10 min. After cooling the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). The Diclofenac was used as standard drug. The results were tabulated in Table.4

$$\% \text{ of inhibition} = \frac{Abs_{660\ (Control)} - Abs_{660\ (Test)}] * 100}{[Abs_{660\ (Control)}]}$$

Table no:4 Anti-inflammatory Screening of synthesized compounds

Compound	Absorbance value (Mean ± SE)	% of denaturation
Control	0.083	-
Diclofenac Sodium	0.153	84.33%
6a	0.12	44.42%
6b	0.121	46.5%
6c	0.129	55.12%
6d	0.123	47.42%
6e	0.124	49.3%
6f	0.126	51.57%
6g	0.127	52.97%
6h	0.133	58.72%

***In-vitro* Anti-oxidant Screening[17]:**

The synthesized compounds were screened for *in-vitro* anti-oxidant activity using Hydrogen Peroxide Scavenging method. All the compounds and the Standard(Ascorbic acid) were dissolved in DMSO as a solvent - stock solution (100µg/100ml) and from stock solution various concentrations (two fold dilutions) of 50, 100, 200, 400 and 800µg/ml were prepared in different volumetric flasks. To each solution, 2 ml hydrogen peroxide was added and the volume was made to 10 ml with phosphate buffer saline (pH-7.4).A control solution was prepared with DMSO in phosphate buffer saline without drug. The absorbance at 230nm was recorded using U.V spectrophotometer against blank (Phosphate buffer saline). The % inhibition by hydrogen peroxide scavenging activity was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{[Abs_{230\ (Control)} - Abs_{230\ (Test)}] * 100}{[Abs_{230\ (Control)}]}$$

The results were tabulated in Table.5

Table no:5 Anti-oxidant Screening of synthesized compounds

Compound	%Inhibition					IC_{50}
	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	
6a	2.3	10.5	16.3	28.9	36.5	6.6
6b	18.8	27.5	55.1	59.7	68.4	5.67
6c	23.1	35.9	48.53	53.9	68.9	3.37
6d	10.23	13.36	24.6	28.3	49.3	5.22
6e	11.8	19.1	25.7	31.8	42.5	7.29
6f	21.3	36.4	47.9	54.3	67.8	3.03
6g	20.9	31.7	45.2	56.5	66.9	5.06
6h	21.4	34.8	49.3	57.2	68.8	3.25
Ascorbic acid	39.6	45.7	53.9	59.5	68.3	2.55

***In-vitro* Anti-microbial Screening[18]:**

The synthesized compounds were subjected to *in-vitro* Antimicrobial screening by disc plate method for zone of inhibition. The anti-bacterial activity was tested against various Gram positive and Gram negative bacteria and anti-fungal activity against various fungal strains compared with standard drugs, Ciprofloxacin and Ketoconazole using solvent control, DMSO. The results were described in Table.6 and 7.

Table no:6 Antibacterial activity of the synthesized compounds

Compound (100µg/ml)	Mean zone of inhibition (*in mm*)			
	B.subtilis	*S.arues*	*K.pneumonia*	*E.coli*
6a	9	-	7	8
6b	-	10	11	10
6c	12	17	17	13
6d	10	7	9	10
6e	9	10	13	11
6f	13	17	19	13
6g	15	16	15	12
6h	16	17	19	12
Standard	19	21	22	14
Control	-	-	-	-

Table no:7 Antifungal activity of the synthesized compounds

Compound	Mean zone of inhibition (*in mm*)			
	Aspergillus flavus		*Aspergillus niger*	
6a	6	9	7	10
6b	8	12	9	11
6c	11	13	11	14
6d	7	8	6	10
6e	6	9	7	11
6f	11	13	10	13
6g	7	14	8	14
6h	7	13	9	13
Ketoconazole	11	13	15	17
Control	6	9	7	10

RESULTS AND DISCUSSION

All the synthesized compounds were first purified by successive recrystallisation using appropriate solvents. The synthesized compounds were characterized, subjected to spectral analysis such as IR, ^{1}H-NMR and were screened for anti-diabetic, anti-inflammatory, anti-oxidant and anti-microbial activities.

Out of these synthesized compounds, **6c, 6f, 6g and 6h** showed significant activity and others showed moderate activity over biological activities.

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