International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.7, pp 1970-1975, November 2014

Anthelmintic and Anti-microbial activities of synthesized heterocyclic pyrazole and its derivatives from fluoro substituted hydrazino benzothiazole

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Abstract : A seven new series of Pyrazole heterocyclic compounds and its derivatives are synthesized by reacting substituted α-β unsaturated carbonyl compound(i.e Chalcones) with prepared fluoro substituted 2-hydrazino 1,3-benzothiazole (from fluoro substituted 2-aminobenzothiazole with hydrazine hydrate) under conventional method. The structure & properties for compounds was determined by Physical and spectral data like solubility, M.P, IR by KBr method, ^{1}H-NMR & Mass spectroscopy, and all the obtain compounds are evaluated for their In-vitro anthelmintic and anti-microbial activity by standard methods.
Key words: Chalcones, 2-hydrazino benzothiazole, Pyrazole, anthelmintic and anti-microbial activity.

Introduction

Pyrazole heterocyclic compounds represent important building blocks in organic and medicinal chemistry. Many significant research activities were carried out towards this structure. The heterocyclic pyrazole and its derivatives shows remarkable biological and pharmacological activities as antitumor [1], antibacterial, antifungal, anti-inflammatory[2,3,4,5], anti-oxidant[6], antidepressant & anticonvulsant[7], analgesic[8], antihistaminic[9,10,11,12] and so on.

The 2-amino benzothiazole scaffold is favored structure in medicinal chemistry which showing numerous biological activities such as antimicrobial[13], anti-inflammatory[14], anthelmintic[15], anticancer[16,17], anti-tubercular[18], anti-diabetic[19], anthelmintic[20,21] so on. in the present work 6-fluoro-2-amino benzothiazole was prepared and converted to 6-fluoro-2-hydrazino benzothiazole which needed for cyclization.

Chalcones have played a crucial part in the development of theory of heterocyclic compounds, and also they used extensively in organic synthesis [22,23,24] . A classical synthesis of these compounds involves the base-catalyzed aldol condensation reaction of ketones and aldehyde to give *α,β*-unsaturated ketones (chalcones) in the presence of PEG-400 as catalyst to speed up the reaction and can easily removed from the sample by simple washing.

It must be emphasized that combination of 6-fluoro-2-hydrazino benzothiazole with substituted chalcones is a well known approach to design new drug molecules, which allows achieving new pharmacological profile, increased action potency with lowered toxic nature. These two reacting mixture undergo subsequent cyclization to afford pyrazoles and its derivatives.

Experimental

The chemicals used in the synthesis of above titled compounds are obtaining from S.D. fine, Fischer syntifics, Qualigens & Merck chemicals. The melting point for the compounds were determined by open capillary method which are incorrect, Analytical elemental studies, all the synthesized compounds are characterized and identified by FT-IR by KBr method using ANALYTICAL TECHNOLOGIES FT-IR spectrophotometer 2202. Some selected compounds were subjected to ^{1}H-NMR spectra data were recorded on Bruker 300 MHz in DMSO and FAB-Mass for structural confirmation, all the compounds are screened for *in-vitro* anthelmintic and antimicrobial activities and the results are shown in the tables.

Methadology

Synthesis of Substituted chalcones 1a-g :

An Equimolar concentration of *p*-hydroxy acetophenone (of 0.01mole) and *p*-chloro benzaldehyde (of 0.01mole) was taken in 250ml RBF, to which 30ml of distilled alcohol was added along with 2ml of catalytic PEG-400. The mixture was stirred on ice bath at 0-10^{0}c with constantly adding about 10-15ml of 20% NaOH for 10min, after completion stirring was continued for another 90min at room temperature and left overnight, the resulting mixture was poured into 100ml crushed ice stirred and neutralized with dilute HCl drop wise to precipitate. Product was filtered, dried and re-crystallized from absolute alcohol. By this the other 6 different chalcones are prepared in the same manner.

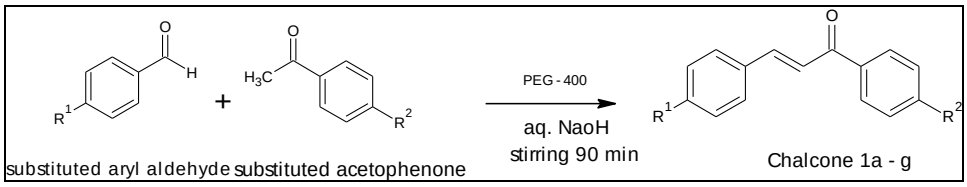
Synthesis of 6-fluoro-2-Hydrazino-1,3-benzothiazole 2 :

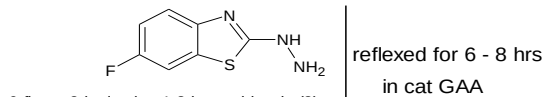
To an 8ml conc. HCl added drop wise with stirring into an 8ml hydrazine hydrate taken in 250ml RBF and maintain the temperature between 5-10^{o}C. To this about 30ml of ethylene glycol and (0.1mole) 6-fluoro-2-amino benzothiazole was added and stirring was continued for 30min. Then the reacting mixture was refluxed for 6 hrs in a heating mantle till the solid crystals appears, cool and pour the reacting mixture into an 250ml crushed ice to obtain the desired product, filtered and dried it.

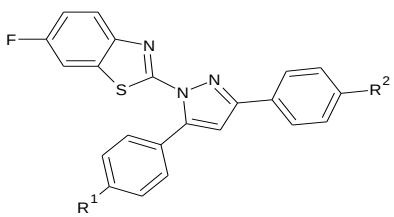
Synthesis of Substituted Pyrazole heterocyclic compounds 3a-g :

In a 250ml RBF take 30ml of absolute alcohol and to which (0.01mole) of 6-fluoro-2-hydrazino-1,3-benzothiazole and (0.01mole) of substituted Chalcone was added . The mixture was refluxed on heating mantle, after 30 min a catalytic glacial acetic acid was added and the condensation was carried out for 6-8 hrs, the reaction time was considered by performing TLC to obtain single spot of reaction. Cool the reacting mixture and transfer it in to 200ml of cold water to precipitate out the product. Wash the product twice in cold water and filtered it, dried and re-crystallized from hot methanol.

General Scheme:







Substituted-2-(3,5-diphenyl-1H-pyrazol-1-yl)-6-fluoro-1,3-benzothiazole (3)

R^{1} = Cl, OCH_3, Furan , R^{2} = OH, NH_2, OCH_3, CH_3, Cl

Properties of synthesized compounds:

P.B-1: 4-[5-(4-chlorophenyl)-1-(6-fluoro-1,3-benzothiazol-2-yl)-1H-pyrazol-3-yl]phenol - 3(a):

Mol Formula- $C_{22}H_{13}ClFN_3OS$, Mol wt- 422, % Yield- 74.72%, mp- 118^o C, *I.R. Spectra* (KBr pellet) υ max cm^{-1} : 600(C-S), 1040(C-F), 1540(C=N), 1235(C-N), 1590(C=C), 810(C-Cl), 3092(C-H), 3350(Ar-OH). ^{1}H-NMR(300 MHz)(DMSO) δ (ppm): Pyrazole CH(1H,s,3.36) Ar-OH(1H,s,6.17) Ar-H(11H,m,6.6-7.96). M/z = 421. Analytical Calculated for $C_{22}H_{13}ClFN_3OS$: C(62.63%)H(3.11%)Cl (8.40%)F(4.50%)N (9.96%)O (3.79%) S(7.60%).

P.B-2: 4-[5-(4-chlorophenyl)-1-(6-fluoro-1,3-benzothiazol-2-yl)-1H-pyrazol-3-yl]aniline –3(b):

Mol Formula- $C_{22}H_{14}ClFN_4S$, Mol wt- 420, % Yield- 81.16%, mp- 110^o C, *I.R. Spectra* (KBr pellet) υ max cm^{-1} : 600(C-S), 1030(C-F), 1545(C=N), 1230(C-N), 1600(C=C), 830(C-Cl), 3090(C-H), 3345(Ar-NH_2). Analytical Calculated for $C_{22}H_{14}ClFN_4S$: C(62.78%) H(3.35%) Cl(8.42%) F(4.51%) N(13.31%) S(7.62%).

P.B- 3: 2-[5-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazole-3(c):

Mol Formula- $C_{23}H_{15}ClFN_3OS$, Mol wt- 436, % Yield- 68.22 %, mp- 134^o C, *I.R. Spectra* (KBr pellet) υ max cm^{-1} : 594(C-S), 1035(C-F), 1530(C=N), 1230(C-N), 1600(C=C), 815(C-Cl), 3055(C-H), 1255(Ar-OCH_3). Analytical Calculated for $C_{23}H_{15}ClFN_3OS$: C(63.37%) H(3.47%) Cl(8.13%) F(4.36%) N(9.64%) O(3.67%) S(7.36%).

P.B-4: 2-[3-(4-chlorophenyl)-5-(2-furyl)-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazole -3(d):

Mol Formula- $C_{20}H_{11}ClFN_3OS$, Mol wt- 396, % Yield- 62.52 %, mp- 114^o C, *I.R. Spectra* (KBr pellet) υ max cm^{-1} : 595(C-S), 1030(C-F), 1540(C=N), 1230(C-N), 1594(C=C), 830(C-Cl), 3098(C-H), 750(furan).^{1}H-NMR(300 MHz)(DMSO) δ (ppm): Pyrazole CH(1H,s,3.36), Ar-H(7H,m,6.7-8.24), Furon(3H,d,6.58ppm). M/z = 396. Analytical Calculated for $C_{20}H_{11}ClFN_3OS$: C(60.69%) H(2.80%) Cl(8.96%) F(4.80%) N(10.62%) O(4.04%) S(8.10%).

P.B-5: 4-[1-(6-fluoro-1,3-benzothiazol-2-yl)-5-(4-methoxyphenyl)-1H-pyrazol-3-yl]phenol-3(e):

Mol Formula- $C_{23}H_{16}FN_3O_2S$, Mol wt- 418, % Yield- 58.12 %, mp- 126^{o}C, *I.R. Spectra* (KBr pellet) υ max cm^{-1} : 610(C-S), 1025(C-F), 1520(C=N), 1232(C-N), 1608(C=C), 1228(Ar-OCH_3), 3080(C-H),3375(Ar-OH). ^{1}H-NMR(300 MHz)(DMSO) δ (ppm): Pyrazole CH(1H,s,3.69)Ar-OCH3(3H,s,3.37), Ar-H(11H,m,6.9-8.06), Ar-OH(1H,s,6.6). M/z = 416. Analytical Calculated for $C_{23}H_{16}FN_3O_2S$: C(66.17%) H(3.86%) F(4.55%) N(10.07%) O(7.67%) S(7.68%).

P.B-6: 6-fluoro-2-[5-(4-methoxyphenyl)-3-(4-methylphenyl)-1H-pyrazol-1-yl]-1,3-benzothiazole-3(f)

Mol Formula- $C_{24}H_{18}FN_3OS$, Mol wt- 416, % Yield- 63.18 %, mp- 117^o C, *I.R. Spectra* (KBr pellet) υ max cm^{-1} : 590(C-S), 1040(C-F), 1520(C=N), 1230(C-N), 1590(C=C), 1295(Ar-OCH_3). Analytical Calculated for $C_{24}H_{18}FN_3OS$: C(69.38%) H(4.37%) F(4.57%) N(10.11%) O(3.85%) S(7.72%).

P.B-7: 2-[3-(4-chlorophenyl)-5-(4-methoxyphenyl)-1H-pyrazol-1-yl]-6-fluoro-1,3-benzothiazole-3(g)

Mol Formula- $C_{23}H_{15}ClFN_3OS$, Mol wt- 436, % Yield- 72.32 %, mp- 122^{o}C, *I.R. Spectra* (KBr pellet) υ max cm^{-1} : 625(C-S), 1043(C-F), 1527(C=N), 1225(C-N), 1602(C=C), 1288(Ar-OCH_3), 3055(C-H),826(C-Cl). ^{1}H-NMR(300 MHz)(DMSO) δ (ppm): Pyrazole CH(1H,s,3.82)Ar-OCH3(3H,s,3.30), Ar-H(11H,m,6.6-8.2). M/z = 435. Analytical Calculated for $C_{23}H_{15}ClFN_3OS$: C(63.37%) H(3.47%) Cl(8.13%) F(4.36%) N(9.64%) O(3.67%) S(7.36%).

Biological Activities:

Anthelmintic activity:

The synthesized compounds are screened for Anthelmintic activity by using Mathew *et al* method and Indian adult earthworms (***Pheretima Posthuma***). The earthworms (collected from the water logged areas of soil in and around Tiruchanoor, Tirupati) were washed with normal saline to remove all faecal materials. The earthworms in 4 - 5 cm in length and 0.1 - 0.2 cm in width were used for all experimental protocol. The

earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study anthelmintic activity.

Five earthworms of nearly equal size were placed in standard drug solution and test compounds solutions at room temperature. Normal saline used as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl sulfoxide (DMSO) and adjusted the volume up to 15 ml with normal saline solution to get the concentration of 0.1 % w/v, 0.2 % w/v and 0.5% w/v. Albendazole was used as a standard drug. The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive.

The mean lethal time for paralysis and death of the earthworms for different test compounds and standard drug are tabulated in table: 01

Table – 01 Anthelmintic activity of synthesized compounds

Sl.no	Compound code	% Concentration in w/v	*Average Time in Minutes*	
			Paralysis Time(min)	**Death Time (min)**
1	CONTROL	0.9 %	-	-
2	ALBENDAZOLE	0.1 % 0.2 % 0.5 %	39 31 21	43 37 25
3	PB-1	0.1 % 0.2 % 0.5 %	74 23 20	77 27 25
4	PB-2	0.1 % 0.2 % 0.5 %	75 24 45	79 29 49
5	PB-3	0.1 % 0.2 % 0.5 %	71 76 19	75 80 25
6	PB-4	0.1 % 0.2 % 0.5 %	44 55 44	50 61 51
7	PB-5	0.1 % 0.2 % 0.5 %	43 47 46	48 52 53
8	PB-6	0.1 % 0.2 % 0.5 %	16 21 17	21 28 24
9	PB-7	0.1 % 0.2 % 0.5 %	43 38 18	49 44 24

Anti-microbial activity:

All synthesized compounds were screened for *In vitro* antibacterial activity against two strains of microorganisms namely ***Staphylococcus aureus*** *(Gram+ve) and* ***Escherichia coli*** *(Gram-ve)*. All those compounds screened for antibacterial activity were also tested for their antifungal activity by using Agar diffusion cup plate method. The fungi employed for screening were: ***Aspergillus Niger*** and ***Candida albicans***. All compounds subjected for antimicrobial activity was performed by two different concentrations and known antibiotics like Ampicillin and Fluconazole were used for comparison purpose respectively. The zone of inhibition was recorded by using zone reader in mille meter (mm) and the results are shown in the Table: 2.

Table: 2 Anti-microbial activity of synthesized compounds

Sl. No	Sample code & Standard	Mean zone of inhibition in (mm)							
		Anti-Bacterial				*Anti-fungal*			
		*Staphylococcus aureus (*G+ve)		*Escherichia Coli* (G^{-ve})		*Candida albicans*		*Aspergillus Niger*	
		50 µg	100µg	50µg	100µg	50 µg	100µg	50µg	100µg
1	Ampicillin	20	24	18	21	-	-	-	-
2	Fluconazole	-	-	-	-	18	21	16	20
3	PB-1	15	19	16	16	15	17	12	16
4	PB-2	17	20	17	20	16	19	15	17
5	PB-3	16	19	16	17	17	18	16	19
6	PB-4	17	22	16	19	16	20	16	18
7	PB-5	18	22	18	19	17	19	16	17
8	PB-6	17	21	16	17	15	17	15	17
9	PB-7	16	20	17	19	16	18	15	16
10	Control	-	-	-	-	-	-	-	-

Result and Conclusion:

As mentioned in the title a new series of substituted *2-(3,5-diphenyl-1H-pyrazol-1-yl)-6-fluoro-1,3-benzothiazole* (i.e Pyrazole containing fluoro substituted benzothiazole) by the condensation of substituted α-β unsaturated carbonyl compound that is Chalcones with 6-fluoro-hydrazeno benzothiazole in ethanolic medium under catalytic GAA, the compounds obtained are re-crystallized and subjected for physical and chemical examination, all compounds structure was confirmed by spectral interpretation studies like I.R, and few selected compounds are subjected to ^{1}H-NMR & Mass to confirm the structure. All the synthesized compounds are evaluated for anthelmintic and antimicrobial activity by standard procedure with slight modification and the results are compared with a known standard compounds. In Anthelmintic activity compounds are prepared as per procedure and Indian earth worm i.e ***Pheretima Posthuma*** which resembles the intestinal live stocks are collected and grouped 5 worms in test, control and standard. Albendazole was used here as standard the paralysis time followed with death time was recorded the compound P.B-6,7,5,4 shows prominent action as compared with test and remaining shows mild to moderate. The compounds used in anthelmintic are also tested for antimicrobial activity by Agar defused cup plate mean zone of inhibition method the bacterial strains used here are ***S.aureus and E.coli*** and fungal strains are ***C.albicans and A.niger*** the test compounds readings are compared with Ampicillin standard drug for bacterial and Fluconazole drug for fungal which are shown in table. Compound P.B-5,4,6,7 shows prominent action on Gram +ve bacteria and compound P.B-5,6,2,4 and 7 shows against gram –ve bacteria, for fungal compound P.B-5,3,2,4 shows good to moderate activity and all other compounds shows mild antibacterial activity. By the result obtained we are planning to do further studies on this series of compounds.

Acknowledgement:

The Author and co-workers are hearty thankful to Smt.P.Sulochana garu chair person Sri Padmavathi school of pharmacy for encouraging and providing enough facility to conduct research activities to upgrade research knowledge in students and faculty.

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