In-Vitro Anti-diabetic & Anti-cancer Investigation of Newly Synthesized Substituted Nitrogen Containing Azoles Analogues

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ABSTRACT

New Molecule with promising anti-diabetic and anticancer properties are being sought after, a set of Substituted Substituted Nitrogen Containing Heterocyclic analogues were synthesized, which were constructed by various material like thiazole, pyrazole, pyrimidine and bio-assayed. Synthesized molecules be confirmed with diverse modern investigative method like FT-Infrared, Proton NMR, Carbon 13 NMR & Mass spectrometry data. The entire prepared molecule is screen to investigate their in-vitro anti-diabetic and anti-cancer activity. Finally, the anticancer activity was found by brine shrimp lethality bioassay and allium cepa root tip meristem model. Anti-diabetic activity was carried out by glucose diffusion inhibitory study. From the results, most of the compounds exhibited good activities.

Keywords: Thiazole, Pyrazole, Pyrimidine, Synthesis, Characterization, In-vitro Anti-diabetic and Anti-cancer Activities

INTRODUCTION:

Numerous synthetic organic compounds have evolved into crucial medicines as a result of the ongoing hunt for better treatments or substrates. The building blocks of medicinal chemistry are regarded as heterocyclic molecules. It was anticipated that the varied group structures and hetero atoms present in heterocyclic compounds would increase the various functions.Due to the extensive range of pharmacological activities that heterocyclic compounds are capable of, they have attracted a great deal of attention during the past few decades. In addition to that, it has been shown that N2 and Sulphur heteroatoms that comprised heterocyclic compounds with five or six members play a significant role in therapeutic purposes [1–5]. In particular, it has recently been found that the nuclei of indole, pyrimidin, thiazole, and pyrrazine have therapeutic properties that include, among others, anticancer, antifungal, antitubercular, antidiabetic, antibacterial, anti-inflammatory, antiviral, antimicrobial, and antiproliferative agents. Chemical analysts and biologists have been working consistently to design and produce heterocyclic hybrids over time due to their importance for medicine[6].Thiazole moieties are the sulphur-nitrogen containing heterocyclic compounds that ply an important role in designing a new series of structural entities for pharmacological screening. Thiazole and fused thiazole ring systems have invited immense attention from medicinal chemistry researchers due to their wide variety of pharmacological activities like antimicrobial,

antitubercular, antitumor, antihelmintic, analgesic, anti-inflammatory, antimalarial, and anticonvulsant activities [7-8].

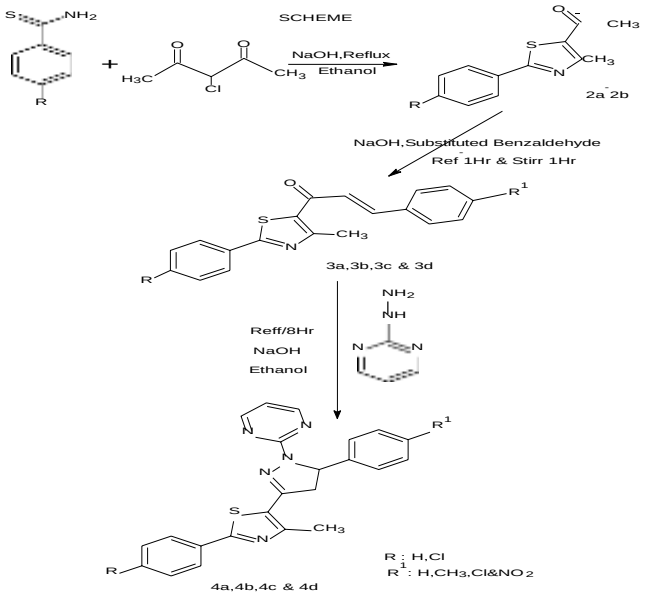
The cancer is an exceedingly proliferative disease that can straighten out too many organs. Cancer is the second main cause of death in the world. Presently used chemotherapeutic agents restrain the growth of tumour through suppression of DNA replication and transcription. Nonetheless, the aim of drug discovering new curative anticancer agents in last decade has led to targets of specified molecular modifications in tumour cells. The new approach has targeted on the development of small biologically active molecules containing significant activity beyond toxicity related to the usual chemotherapy[9-10]. The Diabetes has been on ascent world over, but a major healthproblem and social burden in developing countries such as India. A prospective source of new antidiabetic medication discovery, novel diagnostic, therapeutic, and preventative approaches are thus urgently needed for cancer therapy. Research in this direction is still going on, to find an alternative drug which can target the malignant tissues, less toxic and affordable by common man.similarly New types of antidiabetic agents are continually needed with diabetes becoming the epidemic in the world. So in this research an effort to develop safe and potent anti-diabetic and anticancer agents

EXPERIMENTAL SECTION

Material and Methods:

All chemicals of LR grade were used for synthesis, which were procured from Sigma-Aldrich, Merck, SD fine and Aware company. The solvents of LR grade were used and purified before use.

The Fourier-transform infrared spectroscopy (FT-IR) absorption spectra were obtained in the range 4000-400 cm-1 on Alpha Bruker FT-IR instrument. ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on BrukerUx-NMR instrument and the samples was made in CDCl3 using methyl silane (Me4Si) as the internal standard and chemical shifts were expressed in parts per million (PPM) and the melting point (MP) of the all the newly synthesized compounds were recorded on Metler Fp-51 instrument.



sExperimental Procedure.

Synthesis of 1-(2-substitutedphenyl-4-methylthiazol-5-yl) ethanone (2a-2b). Equimolar 3-chloropentane-2,4-dione was refluxed for 6-7 hours with substituted phenyl thiamide in 75 to 100ml of methanol/ethanol.With the aid of a thin layer chromatograph (TLC), the intermedia tes created after each step of the reaction were assessed.The mixture was decanted into ice-cold water after the reaction was finished.Filtering and recrystallizing the obtained precipitate s in alcohol.

Synthesis of 1-(4-methyl-2-substituted phenylthiazol-5-yl)-3-substituted phenylprop-2-en-1-one. (3a-d): QS ml of ethayl Alcohol were used to dissolve a mixture of substituted arylaldehyde and substituted 1-(2-substitutedphenyl-4-methylthiazol-5-yl) ethanone (1 mmol each). 1 cc of newly made, 10% sodium hydroxide solution was added to the reaction mixture. At 80–90°C, the reaction mixture was refluxed. TLC was utilised to monitor the reaction's progress. The reaction mixture was placed into ice-cold water and agitated for 15 minutes after the reaction had finished. The resulting solid, which was yellow in colour, was filtered before being rinsed with cold water and dried. To obtain pure named component (3a-d), the crude product was recrystallized with ethyl Alcohol.

Compound.3a: 1-(4-methyl-2-phenylthiazol-5-yl)-3-phenylprop-2-en-1-one. M.P. 125^{o}C, Mol. Formula: $C_{19}H_{15}NOS$, Yield.68%. IR (ν cm−1): 3039(C-H *Str*, Ar), 2965(C-H *Str*), 1703(C=O, *Str*), 1621(C=N, *Str*), 1503(C=CH *Str*).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.283-8.0231(d,t, 10H, Ar-H), 7.4232-7.3982(dd, 1H, -CO-CH=CH), 6.985-7.0322(dd, 1H, -Ar-CH=CH), 1.8982(s, 3H, Methyl group in thiazole). Mass (LC-MS): m/z 305.09(M), 306.21(M + 1, 100%).

Compound.3b:1-(4-methyl-2-phenylthiazol-5-yl)-3-(p-tolyl)prop-2-en-1-one. M.P. 168^{o}C, Mol. Formula: $C_{20}H_{17}NOS$, Yield.76%. IR (ν cm−1): 3065(C-H *Str*, Ar), 2991(C-H *Str*), 1712(C=O, *Str*), 1632(C=N, *Str*), 1512(C=CH *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.2901-7.8643(d, t, 9H, Ar-H), 7.4231-7.4093(dd, 1H, -CO-CH=CH), 6.9909-7.0021(dd, 1H, -Ar-CH=CH), 2.0943-2.0012(s, 6H, Methyl group). Mass (LC-MS): m/z 319.10(M), 311.32(M + 1, 100%).

Compound.3c: 1-(4-methyl-2-phenylthiazol-5-yl)-3-(4-chlorophenyl) prop-2-en-1-one. M.P. 203^{o}C, Mol. Formula: $C_{19}H_{14}N_2O_3SCl$, Yield.73%. IR (ν cm−1): 3077(C-H *Str*, Ar), 2976(C-H *Str*), 1703(C=O, *Str*), 1621(C=N, *Str*), 1518(C=CH *Str*), 789(C-Cl *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.3894-7.7676(d, t, 9H, Ar-H), 7.3894-7.3001(dd, 1H, -CO-CH=CH), 6.9021-7.1097(dd, 1H, -Ar-CH=CH), 1.9843(s, 3H, Methyl group). Mass (LC-MS): m/z 339.05(M), 340.03(M + 1, 100%), 341.26(M + 2, 30%).

Compound.4d: 1-(4-methyl-2-phenylthiazol-5-yl)-3-(4-nitrophenyl)prop-2-en-1-one. M.P. 156^{o}C, Mol. Formula: $C_{19}H_{14}N_2O_3S$, Yield.70%. IR (ν cm−1): 3087(C-H *Str*, Ar), 2978(C-H *Str*), 1706(C=O, *Str*), 1632(C=N, *Str*), 1521(C=CH *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.4982-7.5647(d, t, 9H, Ar-H), 7.4002-7.3987(dd, 1H, -CO-CH=CH), 6.9908-7.0021(dd, 1H, -Ar-CH=CH), 1.8974(s, 3H, Methyl group). Mass (LC-MS): m/z 350.10(M), 351.25(M + 1, 100%).

Synthesis of -methyl-2-substitutedphenyl-5-(5-substituted-phenyl-1-(pyrimidin-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)thiazole.(4a-d): When combined with 2-

hydrazineylpyrimidine (0.01M), the product 3a-d (1-(4-methyl-2-phenylthiazol-5-yl)-3-substituted phenylprop-2-en-1-one) (0.01M) was dissolved in ethanol. A modest amount of distilled water was used to prepare the aq. base solution (0.02M) from KOH. The mixture used in the reaction was refluxed for 7-8 hours, cooled, diluted with water, and acidified with concentrated HCl. From ethanol, the product was filtered, dried, and crystallised.

4a:4-methyl-2-phenyl-5-(5-phenyl-1-(pyrimidin-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)thiazole. M.P. 185^{o}C, Mol. Formula: $C_{23}H_{19}N_5S$, Yield.80%. IR (ν cm−1): 3079(C-H *Str*, Ar), 2988, 2870, 2741(C-H *Str*, Aliphatic), 2320(-C-S-C *Str*, in thiazole), 1617(C=N, *Str*), 1533(C=CH *Str*), 1329(C=C *Str*), 1050(C-N, *Str*), ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.1092-8.0984(d, 2H, Ar-H), 7.8943-7.8110(d, 2H, Ar-H), 7.7093-7.6984(d, 2H, Ar-H), 7.3744-7.2822(t, 6H, Ar-H), 7.1932(t, 1H, Ar-H), 4.5192-4.5003(dd, 2H, Pyrazole), 3.1290(t, 1H, Pyrazole), 1.9781(s, 3H, Methyl group in thiazole). Mass (LC-MS): m/z 397.14(M), 398.03(M + 1, 100%).

4b:4-methyl-2-phenyl-5-(1-(pyrimidin-2-yl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl) thiazole.. M.P. 206^{o}C, Mol. Formula: $C_{24}H_{21}N_5S$, Yield.78%. IR (ν cm−1): 3089(C-H *Str*, Ar), 2977, 2866, 2739(C-H *Str*, Aliphatic), 2333(-C-S-C *Str*, in thiazole), 1629(C=N, *Str*), 1531(C=CH *Str*), 1321(C=C *Str*), 1063(C-N, *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.3094-8.2983(d, 2H, Ar-H), 8.1988-8.0092(d, 2H, Ar-H), 7.7872-7.7032(d, 2H, Ar-H), 7.3923-7.3012(d, 2H, Ar-H), 7.2201-7.1764(t, 3H, Ar-H), 6.8743(t, 1H, Ar-H), 4.5986-4.5209(dd, 2H, Pyrazole), 3.0832(t, 1H, Pyrazole), 2.0943(s, 3H, Methyl group in thiazole), 1.8934(s, 3H, Methyl group in thiazole).Mass (LC-MS): m/z 411.15(M), 412(M + 1, 100%).

4c:5-(5-(4-chlorophenyl)-1-(pyrimidin-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-4-methyl-2-phenylthiazole. M.P. 229^{o}C, Mol. Formula: $C_{23}H_{18}N_5ClS$, Yield.79%. IR (ν cm−1): 3099(C-H *Str*, Ar), 2989, 2854, 2745(C-H *Str*, Aliphatic), 2323(-C-S-C *Str*, in thiazole), 1632(C=N, *Str*), 1527(C=CH *Str*), 1319(C=C *Str*), 1055(C-N, *Str*), 799(C-Cl, *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.4775-8.4102(d, 2H, Ar-H), 8.2537-8.1982(d, 2H, Ar-H), 7.8723-7.8043(d, 2H, Ar-H), 7.4923-7.3032(d, 2H, Ar-H), 7.0222-7.0001(t, 3H, Ar-H), 6.9843(t, 1H, Ar-H), 4.6543-4.5994(dd, 2H, Pyrazole), 3.1974(t, 1H, Pyrazole), 1.9902(s, 3H, Methyl group in thiazole). Mass (LC-MS): m/z 431.10(M), 432(M + 1, 100%), 433(M + 2, 30%).

4d:4-methyl-5-(5-(4-nitrophenyl)-1-(pyrimidin-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-2-phenylthiazole. M.P. 187^{o}C, Mol. Formula: $C_{23}H_{18}N_6O_2S$, Yield.82%. IR (ν cm−1):3012(C-H *Str*, Ar), 2993, 2823, 2715(C-H *Str*, Aliphatic), 2314(-C-S-C *Str*, in thiazole), 1633(-NO_2*Str* , Ar-$NO_{2)}$, 1614(C=N, *Str*), 1545(C=CH *Str*), 1322(C=C *Str*), 1062(C-N, *Str*), ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 8.5083-8.3720(d, 2H, Ar-H), 7.9782-7.8406(d, 2H, Ar-H), 7.7983-7.7813(d, 2H, Ar-H), 7.6971-7.6828(d, 2H, Ar-H), 7.6774-7.5037(t, 1H, Ar-H), 7.4991-7.3998(t, 3H, Ar-H), 4.5313-4.5112(dd, 2H, Pyrazole), 3.1062(t, 1H, Pyrazole), 1.9212(s, 3H, Methyl group in thiazole). Mass (LC-MS): m/z 431.10(M), 432(M + 1, 100%),

Pharmacological activity:

In-vitro Anti-Diabetics-Glucose diffusion inhibitory study: A solution of maltose substrate was incubated at 350°C with a Tris volume of buffer pH 8.0 and various concentrations of lead molecules 4a–d to determine the glucose diffusion inhibitory action. The reaction was started by adding the enzyme -glucosidase to the reaction mixture, which was then given a 350°C incubation period. After that, the reaction was quantified by adding a colorimetric reagent such DNSA [11-12]. As a control, ethanol was made using a similar technique but with lead molecules substituted.The colour compounds' intensity was determined at 540 nm. The following formula was used to calculate the percentage of inhibition (I%).

$$\% \text{ inhibitory activity} = (Ac\text{-}As)/Ac \times 100$$

Where $Abc_{control}$ is the absorbance of the control and $Abs_{sample}$is the absorbance of the sample.

Table 1 : Anti-diabetic activity (Glucose diffusion inhibitory study) of Molecules (4a,4b,4c & 4d) (Scheme-I).

[Sample Name]	[IC_{50} (µg)]
4a	53.7
4b	65.34
4c	56.96
4d	**47.35**
Acarbose	**33.84**

In-vitro Anticancer activity-Brine Shrimp Lethality Bioassay:

In a 1 litre conical-shaped container with continual aeration and sterile artificial sea water, 150 mg of brine prawns (Artemia salina) eggs were incubated for 72 hours. In order to reduce the chance of larvae dying during incubation due to pH lowering, the pH was then increased to 8.5.After 48 hours, feed the larvae by adding 15ml of 0.06% yeast solution to a vessel for every litre of salt water. Hatching takes roughly 72 hours. For the Bioassay, active nauplii that were free of egg shells were gathered and used[26]. In a test tube containing sample, 5 ml of synthetic sea water was added, along with ten active nauplii. Three test tubes with concentrations of 25, 50, and 100 g/ml of the synthetic chemicals 4a–d were used for the experiment. Cyclophosphamide was employed as the standard medication while sea water (the vehicle) served as the control. Live nauplii were counted and the LC50 value was

estimated after 24 hours. By combining the mean number of larvae that survived in the test and control tubes, the percentage of lethality was calculated. LC50 values were calculated using Finney's probit statistical analysis method from concentration versus percentage lethality.

Table.2.: Anti-cancer activity-(Brine Shrimp Lethality Bioassay) of Lead molecule-(4a,4b,4c & 4d) (Scheme-I).

S.No	'Compounds	Concentrations--(µg/ml)	No of -Shrimp tested	Average Mortality after 24hr.	Percent --Mortality	LC50--(µg/ml)
1	**Control**	00	10	00	00	0
2	**4a**	20	10	05	50	31.53
		50	10	05	50	
		100	10	07	70	
3	**4b**	20	10	01	10	92.17
		50	10	04	40	
		100	10	05	50	
4	**4c**	20	10	02	20	179.16
		50	10	02	20	
		100	10	04	40	
5	**46**	20	10	01	10	179.16
		50	10	02	20	
		100	10	03	30	
6	**Cyclophosphamide**	20	10	05	50	21.34
		50	10	06	60	
		100	10	08	80	

RESULTS AND DISCUSSION:

Synthesis: The newly synthesised novel pyrazole, pyrimidine-fused thiazole derivatives (4a-d) demonstrated adequate analyses for the intended structures. In this method of synthetic synthesis, substituted acetophenone and substituted benzaldehyde react to create chalcones (3a-d). The title derivatives (4a-d) were produced through further cyclization reaction with hydrazineylpyrimidine. Spectral data verified each of these formations. By using IR spectroscopy, novel Pyrimidine-fused Thiazole compounds were identified from the spectrum characterisation of the Pyrazole. Practically all synthetic compounds exhibit the predicted aromatic and aliphatic C-H stretching frequencies at about 3001–3098 cm-1 and 2960–2798 cm-1, respectively. There is evidence of -CSC- stretching frequency in all of the compounds' absorption in the range of 2300–2340 cm-1, and all of the compounds exhibit C=C stretching of the aromatic ring at a wavelength of 1501–1565 cm-1, respectively. The area between 783 and 845 cm-1 is clearly absorbed in the Ar-Cl and Ar-F stretching. Every chemical with a -CH3 group exhibits maxima at about 1235 cm-1 and 1025 cm-1, respectively. Similar to this,

the 1HNMR (DMSO-d6) spectra of novel pyrazole derivatives formed from thiazole fusion reveal singlets, doublets, and triplets at 6.8943, 8.5231, and 2.20913, 1.8943 for methyl protons (CH3 group in thiazole ring). All compounds' carbon atoms are most susceptible to replacement.

Acarbose, a -glucosidase inhibitor, lowers the absorption of complex carbs from the gut while also reducing the digestion of these foods for anti-diabetic effects.Due to the presence of electrophilic groups (-NO2) in contrast to the usual medication Acarbose, compound 4d has demonstrated considerable antidiabetic action. Out of all synthesized molecule compound 4d (47.35 g/ml) has the highest IC50 values of all the compounds (Table 1) Since the concentration of the synthesised compounds was discovered to be directly related to their anticancer activity (Brine Shrimp lethality activity), lead molecules were shown to possess this property. When compared to conventional cyclophosphamide (21.32 g/ml), the lead compounds' IC50 values were comparable.

CONCLUSION: Physical and spectroscopic methods were employed to create the novel pyrazole, pyrimidine-fused thiazole derivatives (4a-d) and characterise them. Significant anti-diabetic and anti-cancer properties were present in all of the synthesised compounds.

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