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Synthesis and evaluation of 4-aryl-2-[(2*E*)-2-substituted hydrazinyl]-1,3-thiazoles for possible antioxidant and antimicrobial activities

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ABSTRACT

*In the present work 4-aryl-2-[(2E)-2-(3,4,5-trimethoxybenzylidene)hydrazinyl]-1,3-thiazoles and 4-aryl-2-{(2E)-2-[5-phenylfuran-2-yl)methylidene}hydrazinyl}-1,3-thiazoles were synthesized in good yield by cyclisation of the corresponding thiosemicarbazones with substituted phenacyl bromides in alcohol media. The thiosemicarbazones were in turn prepared by treating aryl aldehydes /arylfurfuraldehydes with thiosemicarbazide in the presence of few drops of sulphuric acid in alcohol media. The structures of newly synthesized compounds were characterized by the spectral studies. The newly synthesized compounds were evaluated for their anti-microbial and antioxidant activity. From the biological studies, it was possible to observe that some of the substituents on the phenyl ring influenced the activity. Among synthesized compound **2b, 4a** and **4f** have shown very good antioxidant activity when compared the reference drug. Compounds showed moderate to good antimicrobial activity at low concentration.*

Keywords: hydrazinyl, thiazole, Schiff's bases, antimicrobial, antioxidant

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INTRODUCTION

Free radicals and active oxygen species have been related with cardiovascular and inflammatory diseases and even with a role in cancer and ageing [1, 2]. Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches, and the field of preventive medicine is experiencing an upsurge of interesting medically useful antioxidants [3, 4]. Recent evidence [5] suggests that free radicals, which are generated in many bioorganic redox processes, may induce oxidative damage in various components of the body (e.g. lipids, proteins and nucleic acids) and may also be involved in processes leading to the formation of mutations. Furthermore, radical reactions play a significant role in the development of life limiting chronic diseases such as cancer, ageing, diabetes, arteriosclerosis and others [6]. On the other hand, the widespread use of antimicrobial agents has resulted in the development of resistance to these drugs by pathogenic microorganisms, causing an increase in morbidity and mortality. Although we have newer less toxic antimicrobial agents that are available for

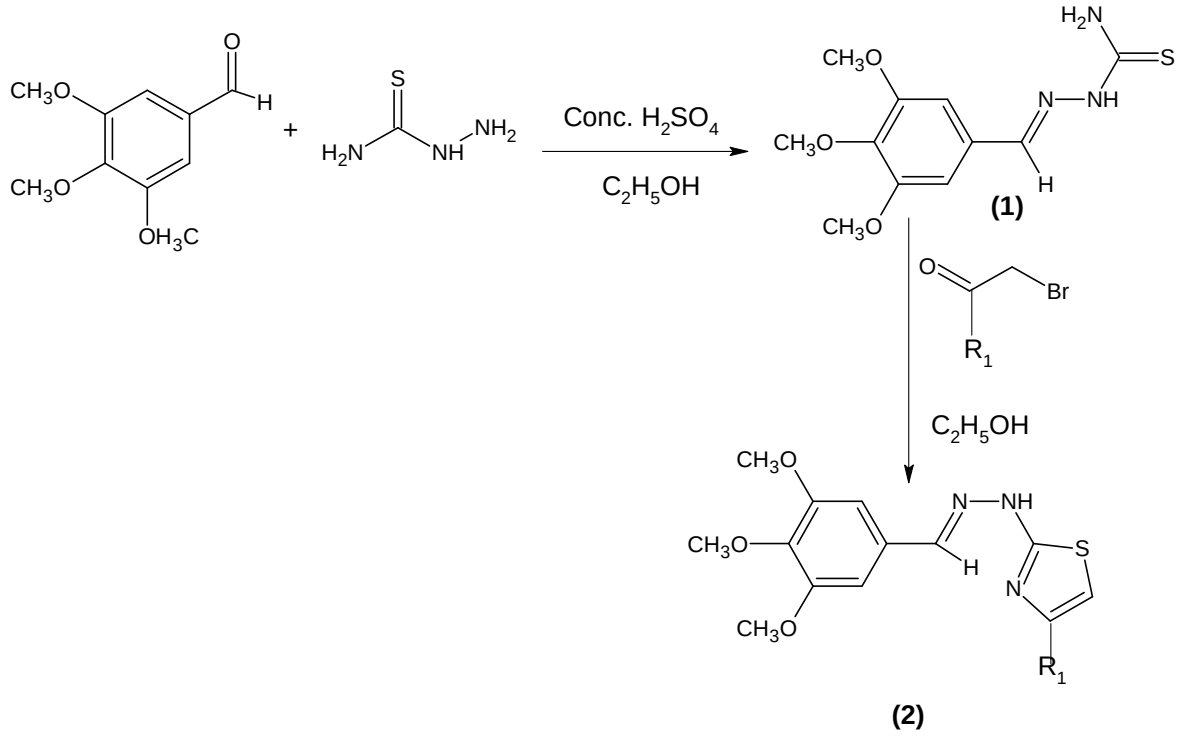
clinical use, their clinical efficacy in some invasive fungal infections, is not optimal [7]. Therefore, the development of new antimicrobial agents is of considerable importance in medicinal chemistry.

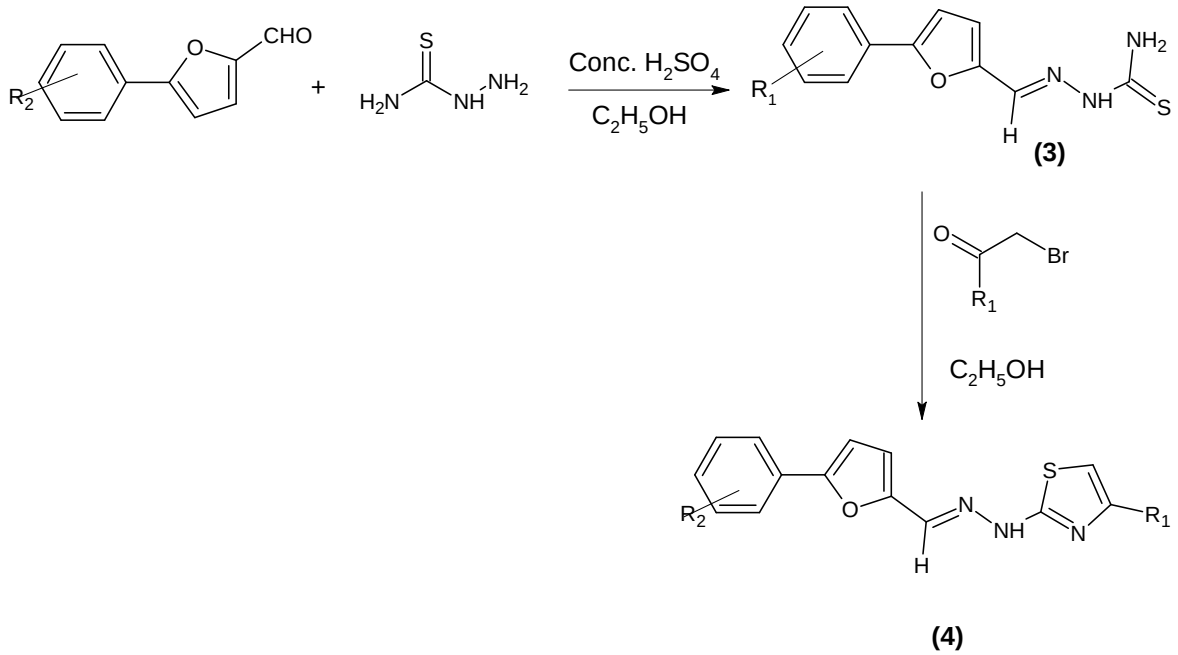
Thiazoles have attracted continuing interest because of their varied biological activities [8], recently found application in drug development for the treatment of allergies [9], hypertension [10], inflammation [11], schizophrenia [12], microbial [13], HIV infections [14], hypnotic and more recently to the treatment of pain [15]. On the other hand Schiff bases have gained importance because of pharmacological and physiological activities associated with them such as antibacterial, antifungal, anticancer and antiviral [16]. Since the thiazole moiety seems to be a possible pharmacophore in various pharmacologically active agents, it has been decided to synthesize compounds with this functionality coupled with Schiff base as possible antioxidant and antimicrobial agents which could furnish better therapeutic results. In view of these marked observations, it was contemplated to synthesize the title compounds and evaluate their biological potency.

MATERIALS AND METHODS

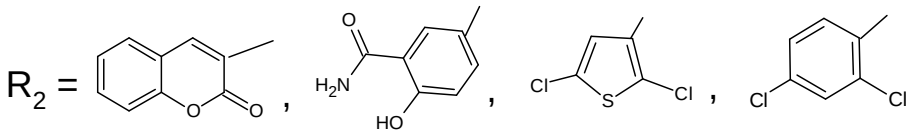
Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded by dispersing the compounds in KBr pellets on a Shimadzu FT-IR 157 spectrophotometer. 1H NMR spectra were recorded on a 400 MHz Bruker Avance spectrometer and all the chemical shift values were reported as δ. The DART-MS was recorded on a JEOL-ACCUTOF JMS-T100LC mass spectrometer having a DART source. Dry helium was used with 4LPM flow rate for ionization at 350^0C. Elemental analysis (CHNS) was performed on the CHNS Elementar Vario EL III. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel plates. All the spectral data of newly synthesized compounds are consistent with proposed structure and microanalysis within ±0.3 of the calculated values.

Substituted 5-aryl-furan-2-carboxaldehydes were prepared through Meerwein reaction [17]. The reaction of substituted acetophenones with bromine afforded required 2-bromo-1-aryl-ethanones [18]. 2,4,6-Trimethoxy benzaldehyde/Substituted 5-aryl-furan-2-carboxaldehydes were treated with thiosemicarbazide to obtain the corresponding thiosemicarbazones [18]. The thiosemicarbazones were further treated with 2-bromo-1-aryl-ethanones to obtain the title compounds **2(a-c)** and **4(a-i).** The synthetic route is outlined in **scheme 1.**





R_1 = 4-Cl, 4-Br, 4-NO_2, 2,4-Cl_2, 3-Cl-4-F, 2-Me-4-NO_2, 2-Me-6-NO_2, 2-OCH_3-4-NO_2, 2,4,5-Cl_3



Scheme 1

Procedure for the preparation of 4-aryl-2-[(2*E*)-2-substituted hydrazinyl]-1,3-thiazoles (2/4)

To a solution of thiosemicarbazone of trimethoxybenzaldehyde/ 5-aryl-furan-2-carboxaldehyde (1mmol) in absolute alcohol media, a solution of 2-bromo-1-aryl-ethanone was added. The mixture refluxed for 4 to 5 hours on a water bath. The mixture was allowed to cool, product formed was filtered, washed and dried and recrystalliized from ethanol.Similar synthetic method was adopted to prepare title compounds and the results are tabulated in **Table 1**.

4-(3-Carboxamido-4-hydroxy-phenyl)-2-(3,4,5-trimethoxybenzylidene)hydrazinyl-[1,3]-thiazole (2b)

Off white solid (yield 84%), mp 178-180^{0}C. IR (KBr, ν in cm^{-1}): 3550 (OH), 3451, 3262, 3165 (NH_2/NH), 2940/2890 (C-H),1670 (C=O), 1612 (C=N), 1585(C=C), 1246 (C-O); ^{1}H NMR (DMSO-d_6, δ): 3.69 (s, 3H, OCH_3), 3.81 (s, 6H, OCH_3), 7.01 (s, 2H, Ar-H), 7.13 (d, 1H, Ar-H, *J* = 8.0 Hz), 7.45 (d, 1H, Ar-H, *J* = 2.0 Hz), 7.48-7.50 (dd, 1H, Ar-H, *J* = 8.0 Hz and 2.0 Hz), 7.72 (s, 1H, thiazolyl-H), 7.98 (s, 1H, N=CH), 8.06 (brs, 2H, NH_2), 12.20 (s, 1H, NH); DART- MS (m/z, %): 428.6 (M^+); Anal. Calcd. for $C_{20}H_{20}N_4O_5S$: C, 56.09; H, 4.72; N,13.11% ; Found: C, 56.06; H, 4.70; N, 13.08%.

4-(2,5-Dichlorothiophen-3-yl)-2-[2-(3,4,5-trimethoxybenzylidene)hydrazinyl]-[1,3]-thiazole (2c)

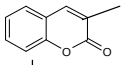
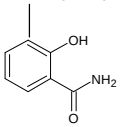
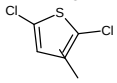
Off white solid (yield 70%), mp 220-222. IR (KBr, ν in cm^{-1}): 3252 (NH), 2938/2888 (C-H), 1590 (C=N), 1230 (C-O), 743 (C-Cl); ^{1}H NMR (DMSO-d_6, δ): 3.69 (s, 3H, OCH_3), 3.82 (s, 6H, OCH_3), 7.00 (s, 2H, Ar-H), 7.49 (s,1H, thienyl-H), 7.65 (s, 1H, thiazolyl-H), 7.96 (s, 1H, N=CH), 12.18 (s, 1H, NH); DART- MS (m/z): 444.7 (M+1); Anal. Calcd. for $C_{17}H_{15}N_3Cl_2O_3S_2$: C, 45.95; H, 3.40; N, 9.46%; Found: C, 45.98; H, 3.42; N, 9.48.

2-[(2-{[5-(4-Bromophenyl)furan-2-yl]methylidene}hydrazinyl]-4-(2,4-dichlorophenyl)-[1,3]-thiazole (4b)

Light yellow solid (yield 70%), mp 176-178^{0}C. IR (KBr, ν in cm^{-1}): 3235 (NH), 2932/2885 (C-H), 1571 (C=N), 753 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.95 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.12 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.17 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.32 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.47-7.49 (dd, 1H, *J* = 8.4 Hz and 2.2 Hz, Ar-H), 7.64 (s, IH, thiazolyl-H), 7.67 (d, 1H, *J* = 2.2 Hz, Ar-H), 7.89 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.95 (s, 1H, N=CH), 12.16

(s, 1H, NH); DART- MS (m/z): 491.7 (M^+), Anal. Calcd. for $C_{20}H_{12}N_3BrCl_2OS$: C, 48.70; H, 2.45; N, 8.52%; Found: C, 48.73; H, 2.48; N, 8.50%.

Table 1: characterization data of compounds (2a-c and 4a-i)

Comp	R_1	R_2	Mol. formula	Melting point (^{0}C)	Yield (%)
2a	_		$C_{22}H_{19}N_3O_5S$	200-202	88
2b	_		$C_{20}H_{20}N_4O_5S$	178-180	84
2c	_		$C_{17}H_{15}Cl_2N_3O_3S_2$	220-222	70
4a	2,4-Cl_2	2,4-Cl_2	$C_{20}H_{11}Cl_4N_3OS$	140-142	89
4b	4-Br	2,4-Cl_2	$C_{20}H_{12}BrCl_2N_3OS$	176-178	70
4c	4-Cl	2,4-Cl_2	$C_{20}H_{12}Cl_3N_3OS$	183-185	83
4d	2-CH_3-4-NO_2	2,4-Cl_2	$C_{21}H_{14}Cl_2N_4O_3S$	124-127	75
4e	4-OCH_3-2-NO_2	2,4-Cl_2	$C_{21}H_{14}Cl_2N_4O_4S$	174-176	71
4f	4-NO_2	2,4-Cl_2	$C_{20}H_{12}Cl_2N_4O_3S$	188-190	77
4g	2,4,5-Cl_3	2,4-Cl_2	$C_{20}H_{10}Cl_5N_3OS$	189-191	68
4h	3-Cl,4-F	2,4-Cl_2	$C_{20}H_{11}Cl_3FN_3OS$	175-177	74
4i	2-CH_3-6-NO_2	2,4-Cl_2	$C_{21}H_{14}Cl_2N_4O_3S$	193-195	69

4-(2,4-Dichlorophenyl)-2-[(2-{[5-(4-chlorophenyl)furan-2-yl]methylidene}hydrazinyl]-[1,3]-thiazole (4c)
Off white solid (yield 83%), mp 183-185^{0}C. IR (KBr, ν in cm^{-1}): 3240 (NH), 2938/2840 (C-H), 1585 (C=N), 748 (C-Cl); ^{1}H NMR (DMSO-d_6, δ): 6.96 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.13 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.20 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.36 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.45-7.47 (dd, 1H, *J* = 8.4 Hz and 2.2 Hz, Ar-H), 7.65 (s, IH, thiazolyl-H), 7.68 (d, 1H, *J* = 2.2 Hz, Ar-H), 7.88 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.97 (s, 1H, N=CH), 12.15 (s, 1H, NH); DART- MS (m/z): 447.3 (M^+); Anal. Calcd. for $C_{20}H_{12}N_3Cl_3OS$: C, 53.55; H, 2.73; N, 9.37%; Found: C, 53.53; H, 2.70; N, 9.36%.

4-(2,4-Dichlorophenyl)-2-[(2-{[5-(4-methoxy-2-nitrophenyl)furan-2-yl]methylidene} hydrazinyl]-[1,3]-thiazole (4e)
Yellow solid (yield 75%), mp 174-176^{0}C. IR (KBr, ν in cm^{-1}): 3244 (NH), 2936/2839 (C-H), 1591 (C=N), 1503/1321 (NO_2), 1243 (C-O), 743 (C-Cl); ^{1}H NMR (DMSO-d_6, δ): 3.67 (s, 3H, OCH_3), 6.95 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.12 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.43-7.45 (dd, 1H, *J* = 8.4 Hz and 2.2 Hz, Ar-H), 7.62 (d, 1H, *J* = 2.2 Hz, Ar-H), 7.65 (s, 1H, thiazolyl-H), 7.86 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.96 (s, 1H, N=CH), 8.01 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.08-8.14 (dd, 1H, *J* = 8.4Hz and 2.4Hz), 8.17 (d, 1H, *J* = 2.4 Hz, Ar-H), 12.13 (s, 1H, NH); DART- MS (m/z): 488.01 (M^+), Anal. Calcd. for $C_{21}H_{14}N_4Cl_2O_4S$: C, 51.54; H, 2.88; N, 11.45%; Found: C, 51.57; H, 2.91; N, 11.43%.

4-(2,4-Dichlorophenyl)-2-[(2E)-2-{[5-(4-nitrophenyl)furan-2-yl]methylidene}hydrazinyl]-1,3-thiazole(4f)
Yellow solid (yield 77%), mp 188-190^{0}C. IR (KBr, ν in cm^{-1}): 3242 (NH), 2940/2852 (C-H), 1582 (C=N), 1522/1311 (NO_2), 757 (C-Cl); ^{1}H NMR (DMSO-d_6, δ): 6.98 (d, 1H, *J* = 3.5 Hz, furyl-H), 7.17 (d, 1H, *J* = 3.5 Hz, furyl-H), 7.51-7.53 (dd, 1H, *J* = 8.3 Hz and 2.4Hz, Ar-H), 7.64 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.65 (s, 1H, thizolyl-H), 7.79 (d, 1H, *J* = 8.3 Hz, Ar-H), 7.82 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.94 (s, 1H, N=CH), 8.18 (d, 2H, *J* = 8.9 Hz, Ar-H), 11.40 (s, 1H, NH); DART- MS (m/z): 458.3 (M^+), Anal. Calcd. for $C_{20}H_{12}N_4Cl_2O_3S$: C, 52.30; H, 2.63; N, 12.20%; Found: C, 52.27; H, 2.65; N, 12.22%.

4-(2,4-Dichlorophenyl)-2-[(2E)-2-{[5-(2,4,5-trichlorophenyl)furan-2-yl]methylidene} hydrazinyl]-1,3-thiazole (4g)
Light yellow solid (yield 68%), mp 189-191^{0}C. IR (KBr, ν in cm^{-1}): 3243 (NH), 2936/2854 (C-H), 1585 (C=N), 748 (C-Cl); ^{1}H NMR (DMSO-d_6, δ): 6.96 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.14 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.40-7.43 (dd, 1H, *J* = 8.2 Hz and 2.1 Hz, Ar-H), 7.59 (d, 1H, *J* = 2.1 Hz, Ar-H), 7.63 (s, 1H, thizolyl-H), 7.84 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.86 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 7.99 (s, 1H, N=CH), 12.18 (s, 1H, NH); DART- MS (m/z): 515.3 (M^+), Anal. Calcd. for $C_{20}H_{10}N_3Cl_5OS$: C, 46.41; H, 1.95; N, 8.12%; Found: C, 46.44; H, 1.98; N, 8.14%.

4-(2,4-Dichlorophenyl)-2-[(2E)-2-{[5-(3-chloro-4-fluorophenyl)furan-2-yl]methylidene}hydrazinyl]-1,3-thiazole (4h)

Light yellow solid (yield 74%), mp 175-177^{0}C. IR (KBr, ν in cm^{-1}): 3252 (NH), 2929/2862 (C-H), 1582 (C=N), 754 (C-Cl); ^{1}H NMR (DMSO-d$_6$, δ): 6.92 (d, 1H, *J* = 3.4 Hz, furyl-H), 7.18 (d, 1H, *J* = 3.4 Hz, furyl-H), 7.39-7.41 (dd, 1H, *J* = 8.2 Hz and 2.4 Hz, Ar-H), 7.52 (d, 1H, Ar-H, *J* = 1.8 Hz), 7.56 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.57-7.62 (m, 1H, Ar-H), 7.65 (s, 1H, thizolyl-H), 7.79 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.75-7.78 (m, 1H, Ar-H), 7.99 (s, 1H, N=CH), 12.20 (s, 1H, NH); DART- MS (m/z): 465.2 (M$^+$), Anal. Calcd. for $C_{20}H_{11}N_3Cl_3FOS$: C, 51.47; H, 2.38; N, 9.00%; Found: C, 51.49; H, 2.40; N, 9.03%.

4-(2,4-Dichlorophenyl)-2-[(2E)-2-{[5-(2-methyl-6-nitrophenyl)furan-2-yl]methylidene} hydrazinyl]-1,3-thiazole (4i)

Yellow solid (yield 69%), mp 193-195^{0}C. IR (KBr, ν in cm^{-1}): 3258 (NH), 2939/2857 (C-H), 1577 (C=N), 1518/1321 (NO_2), 754 (C-Cl); ^{1}H NMR (DMSO-d$_6$, δ): 2.72 (s, 3H, CH_3), 6.93 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.30 (d, 1H, *J* = 3.6 Hz, furyl-H), 7.40-7.42 (dd, 1H, *J* = 8.7 Hz and 2.6 Hz, Ar-H), 7.56 (d, 1H, *J* = 2.6 Hz, Ar-H), 7.65 (s, 1H, thizolyl-H), 7.83 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.93 (d, 1H, Ar-H, *J* = 8.4 Hz), 8.09 (d, 1H, Ar-H, *J* = 8.6 Hz), 8.19-8.21 (dd, 1H, Ar-H, *J* = 8.4 Hz and 8.6 Hz), 8.04 (s, 1H, N=CH), 12.27 (s, 1H, NH); DART- MS (m/z): 472.02 (M$^+$), Anal. Calcd. for $C_{21}H_{14}N_4Cl_2O_3S$: C, 53.29; H, 2.98; N, 11.84%; Found: C, 53.26; H, 3.01; N 11.87%.

Antioxidant Activity (*DPPH radical scavenging assay)*

The DPPH assay was based on the method reported by Kokura *et al.*, 2005 [19]. Briefly, the DMSO solution of compounds at 4000 µg/mL was taken and it was diluted to 5 mL using acetone. To this 0.1ml of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution (10mg/10ml acetone) in acetone was added. The mixed solution was incubated at room temperature for 15 min. The absorbance of stable DPPH was read at 517 nm using UV-Visible spectrophotometer and the remaining DPPH was calculated. Decrease in the absorbance of DPPH solution indicated an increase in the radical scavenging activity. The DPPH solution without sample was used as control .Ascorbic acid was used as reference standard. The experiments were carried out in triplicates. The activity was expressed as percentage DPPH radical scavenging that was calculated from the following equation

$$\text{DPPH scavenging activity (\%)} = \frac{[Ac-As]}{[Ac-Ab]} \text{ X } 100$$

Where *Ac* was the absorbance of the control,*As*for the sample and *Ab*for the blank (MeOH). Each sample was assayed at 4000 µg/mL and all experiments were carried out in triplicate and the % RSC is shown in **Table 2.**

Table 2: DPPH radical scavenging assay for compounds 2a-c and 4a-i

Comp	Percentage inhibition
2a	81
2b	93
2c	24
4a	84
4b	57
4c	77
4d	10
4e	08
4f	88
4g	73
4h	74
4i	55
Ascorbic acid	96

Antibacterial activity:

The newly synthesized compounds were screened for their *in-vitro* antibacterial activity against *Escherichia coli (*ATTC-25922*)*, *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATTC-27853*)* and *Klebsiella pneumonia* (recultured) bacterial stains by serial plate dilution method [20]. Serial dilutions of the drug in Muller Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37^{o}C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth.

A number of antibacterial discs were placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 ^{o}C for an hour. Using a punch, wells were made on these seeds agar plates and minimum inhibitory concentrations of the test compounds in dimethyl sulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using DMSO as a solvent. The Petri dishes were prepared in triplicate and maintained a 37 ^{o}C for 3-4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin as standard. Zone of inhibition was determined for **2a-c** and **4a-i**. The results are summarized in **Table 3**.

The MIC values were evaluated at concentration range, 1.56-25 μg/mL. The figures in the table show the MIC values in μg/mL and the corresponding zone of inhibition in mm.

Table 3: Antibacterial activity of the newly synthesized compounds (2a-c and 4a-i)

Compd	MIC in µg/mL and zone of inhibition in mm			
	S. aureus	***E. coli***	***P. aeruginosa***	***K. pneumoniae***
2a	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
2b	25(<10)	25(<10)	25(<10)	25(<10)
2c	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4a	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
4b	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4c	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
4d	25(<10)	25(<10)	25(<10)	25(<10)
4e	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
4f	25(<10)	25(<10)	25(<10)	25(<10)
4g	25(<10)	25(<10)	25(<10)	25(<10)
4h	25(<10)	25(<10)	25(<10)	25(<10)
4i	25(<10)	25(<10)	25(<10)	25(<10)
Standard (Ciprofloxacin)	1.56(22-30)	6.25(30-40)	6.25(25-33)	6.25(23-27)

Table 4: Antifungal activity of the newly synthesized compounds(2a-c and 4a-i)

Compd	MIC in µg/mL and zone of inhibition in mm			
	P.marneffei	***C. albicans***	***A. flovus***	***A. fumigates***
2a	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
2b	25(<10)	25(<10)	25(<10)	25(<10)
2c	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4a	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
4b	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
4c	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4d	25(<10)	25(<10)	25(<10)	25(<10)
4e	25(<10)	25(<10)	25(<10)	25(<10)
4f	25(<10)	25(<10)	25(<10)	25(<10)
4g	12.5(11-15)	12.5(11-15)	12.5(11-15)	12.5(11-15)
4h	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4i	25(<10)	25(<10)	25(<10)	25(<10)
Standard(Ciclopiroxolamine)	1.56(22-30)	6.25(30-40)	6.25(25-33)	6.25(23-27)

Antifungal activity

Newly prepared compounds were also screened for their antifungal activity against *Aspergilusflavus (*NCIM No. 524*), Aspergilusfumigatus*(NCIM No. 902*), Penicilliummaneffei (*recultured*)* and *Trichophytonmentagrophytes (*recultured*)* in DMSO by serial plate dilution method [21]. Sabourauds agar media was prepared by dissolving peptone (1g). D-Glucose (4g) and agar (2g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of sore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 ^{0}C for 1 h. Wells were made on these seeded agar platesusing a punch. Minimum inhibitory concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 ^{0}C for 3-4 days. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared

with ciclopiroxolamine as standard. Zones of inhibition were determined for **2a-c**and **4a-i**. The results are summarized in **Table 4**.

The MIC values were evaluated at concentration range, 1.56-25 μg/mL. The figures in the table show the MIC values in μg/mL and the corresponding zone of inhibition in mm.

RESULTS AND DISCUSSION

The synthetic pathway for compounds described was achieved by a sequence of reactions starting from aldehydes and is illustrated in scheme 1. The structures of the synthesized compounds were established on the basis of spectral and analytical data. The C, H, N analyses of these compounds are in agreement with the calculated values within the limits of experimental error. The characterization data of the synthesized compounds are presented in Table **1**.

All structures of the title compounds were confirmed by recording their IR, ^{1}H NMR and mass spectra. IR spectrum of **2a** displayed absorption bands at 3224 cm^{-1}, 2932/2836 cm^{-1} for its N-H and C-H stretching vibrations. The spectrum also showed stretching absorption bands at 1712 cm^{-1}, 1575 cm^{-1} and 1125 cm^{-1} for C=O, C=N and C-O-C groups respectively. The absence of the absorption bands corresponding to NH_2 and C=S stretching frequencies of the reactants clearly revealed the formation of thiazoles (**2a-c and 4a-i**). The 400 MHz ^{1}H NMR spectrum of **2a** showed a singlet at δ 3.69 ppm integrating for three protons of the OCH_3 group. Another singlet at δ 3.82 integrating for 6 protons was attributed for the remaining two OCH_3 groups. The two aromatic protons of the trimethoxy phenyl ring resonated as a singlet at δ 6.97 ppm. The single thiazole proton resonated as a singlet at δ 7.76 ppm. A singlet at δ 8.53 ppm was attributed to N=CH proton. A singlet at δ 12.2 ppm was due to NH proton. The chromenone protons resonated as a singlet (δ, 7.98 ppm), a doublet (δ, 7.44, J = 8.4Hz), doublet of doublet (δ, 7.82 - 7.84) and two multiplets (δ, 7.36 - 7.40 and δ, 7.60 - 7.84). Further, DART mass spectrum of **2a** showed a protonated molecular ion peak at m/z 438.2 in conformity with its molecular formula, $C_{22}H_{19}N_3O_5S$.

In the IR spectrum of **4a** the N-H, C-H, C=N and C-Cl groups displayed absorption bands at 3290.9, 3080.3, 1627.9 and 792.2 cm^{-1} respectively. The 400 MHz ^{1}H NMR spectrum of **4a** showed two distinct doublets at δ 6.98 and δ 7.29 integrating for one proton each with coupling constant, J = 3.6Hz attributed to the furan ring protons. Another singlet seen at δ 7.41 was due to the thiazolyl proton. The aromatic protons of two dichlorophenyl rings resonated as three doublets at δ 7.88 (J = 8.4 Hz, 2H), 7.75 (J = 2.0 Hz, 1H), 7.68 (J = 2.2 Hz, 1H) and two doublets of doublets at δ 7.56-7.59 (J = 8.4 Hz/2.4 Hz, 1H) and δ 7.48 – 7.51 (J = 8.4 Hz/2.2 Hz, 1H). A singlet at δ 7.98 ppm was due to N=CH proton. The N-H proton resonated as a singlet at δ 12.3 ppm. Further DART MS spectrum of **4a** showed M+ peak at m/z 481.99 in conformity with its molecular formula $C_{20}H_{11}Cl_4N_3OS$ with its isotopic peaks appearing at m/z 483.99, 485.99, 488 respectively.

Similarly the IR spectrum of compound **4d** showed absorption bands at 3290 cm^{-1} for N-H, 2920/2846 cm^{-1} for C-H, 1575 cm^{-1} for C=N,1514 cm^{-1} for NO_2 (asymmetric str), 1332 cm^{-1} for NO_2 (symmetric str) and 746.0 cm^{-1} for C-Cl. The 400 MHz ^{1}H NMR spectrum of compound **4d** showed a singlet at δ 2.72 ppm attributed to CH_3 protons. Two doublets at δ 7.01 and δ 7.21 ppm integrating for one proton each were attributed to furan protons with coupling constant J = 3.6Hz. The thiazole proton was appeared as a singlet at δ 7.42 ppm. The N=CH proton was also resonated as a singlet overlapped with the doublet of nitro-methyl phenyl at δ 7.97 ppm. The NH proton was appeared as a singlet at δ 12.33 ppm. The dichlorophenyl protons appeared as two doublets and a doublet of doublet at δ 7.88 (J = 8.4Hz, 1H), δ 7.67 (J = 2.2 Hz, 1H), and δ 7.47-7.50 (J = 8.4Hz and 2.2 Hz, 1H). The nitro methyl phenyl protons also resonated as two doublets and a doublet of doublet at δ 7.98 (J = 8.2 Hz, 1H), 8.21(J = 2.2 Hz, 1H) and δ 8.13-8.16 (J = 8.2 Hz and 2.2 Hz, 1H). The DART mass spectrum of **4d** showed protonated molecular ion peak at m/z 473.0 in conformity with its molecular formula. $C_{21}H_{14}Cl_2N_4O_3S$. It also showed isotopic peaks at m/z 475.08 and 477.08.

DPPH radical scavenging activity

The antioxidant activity of a compound is due to several factors and they are (i) suppress the formation of reactive oxygen species by inhibiting the enzyme responsible for its generation(ii) chelate the metal ion responsible for the production of free radical (iii) scavenge the reactive oxygen species (iv) up regulate or protect the antioxidant defense mechanism. Hence the antioxidant potential of any compound is related to its (i) hydrogen or electron donation capacity (ii) its ability to stabilize and delocalize the unpaired electron (iii) potential to chelate transition metal ions. Thus antioxidants in general turn out to be stabilizers counteracting the oxidative degerative processes

mediated by free radicals. There is an increase in the use of methods for estimating the efficiency of substances as antioxidants.

One such method that is currently popular is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH). Here the mechanism of action is due to the transfer of hydrogen atom. The purpose of our study is to examine the percentage inhibition of DPPH when compared with the standard ascorbic acid. When a solution of DPPH is mixed with that of the substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of its violet colour. Representing the DPPH radical by $Z^{\cdot}$ and the donor molecule by AH the primary reaction is

Z. + AH=ZH+A. (1)

Where ZH is the reduced form and A. is the free radical produced in the first step. This latter radical will then undergo further reactions which control the overall stoichiometry that is the number of molecules of DPPH reduced by one molecule of the reductant. The DPPH molecule Z. is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH. The free radical A. evidently then reacts with another molecule of the same kind that was produced by a parallel reaction to (1)

A. + A. = A-A

This therefore leads to the observed reduction of two molecules of DPPH by two molecules of the reductant, that is, a 1:1 stoichiometry.

The DPPH radical scavenging activity in terms of percentage inhibition exhibited by the title compounds are summarized in Table **2**. In this assay compound **2a, 2b, 4a, 4c, 4f, 4g** and **4h** are the most active compounds (73-93%) and have shown significant radical scavenging activity when compared with the reference standard. Among seven of these compounds **2b (**93%) was as active as ascorbic acid, where as compounds **2a, 4a and 4f** showed very good activity. The primary antioxidants consist mainly of hindered phenols and hindered aromatic amines. They scavenge and destroy the chain propagating peroxy and alkoxy radicals before they can react with the polymer. The high activity in **2b** may be attributed to the presence of –OH group in the molecule. Efficient phenolic antioxidants are known to terminate the peroxidation of free radical chains by donating a phenolic hydrogen atom. A high rate for reaction 1 is expected to correlate with a low O–H bond dissociation energy compared with the high bond dissociation energy of N-H bond. The DPPH radical abstracts one of the hydrogen atoms from the antioxidant molecule and gets converted into stable 1, 1-diphenyl-2-picryl hydrazine. The other compounds are showed considerably good activity may be due to the presence of N-H group and the activity is affected by the type of substituent attached to it and the structure. In general, however, the introduction of electron releasing groups to the aromatic ring increases the antioxidant activity, whereas electron withdrawing groups decrease it .Compounds **4b and 4i** have moderate activity (50-60%) and compound **2c, 4d** and **4e** have insignificant (8-24%) activity.

Antimicrobial activity studies

From the antimicrobial results obtained, the structure activity relationship can be drawn for the test compounds **2a-c** and **4a-i**. The results are presented in **Table 3 and 4**. The variation in the antimicrobial activity of the test compounds was explored by varying the substituents. Among the tested compounds **2a**, **2c** and **4b** exhibited maximum antibacterial activity against all the bacterial pathogens at 6.25 µg/mL concentration equivalent to that of the reference drug, ciprofloxacin. The good activity is attributed to the presence of of pharmacologically active chromene nucleus in compound **2a** and thiophene moiety in compound **2c** and bromo group attached to the para position of the phenyl ring. Compounds **4a, 4c** and **4e** showed moderate activity at a concentration of 12.5 µg/mL due to the presence of 4-Cl and 4-OCH_3 groups attached to phenyl ring connected to furan ring. Compounds **2b, 4d, 4f, 4g, 4h** and **4i** did not show any marked activity even at a concentration of 25 µg/mL of the sample.

The investigation of antifungal screening data revealed that compounds **2a, 2c, 4c** and **4h** having chromene nucleus and -Cl substituent on the aromatic ring showed maximum inhibition at 6.25 µg/mL concentration equivalent to that of the reference drug, ciclopiroxolamine used for antifungal screening studies. Compounds **4a, 4b and 4g** showed moderate activity at a concentration of 12.5 µg/mL. However, compounds 2b, 4d, 4e 4f and 4i having substituents like $–CH_3$, NO_2 and $–OCH_3$ on the phenyl ring attached to furan ring did not show significant activity.

CONCLUSION

In conclusion, we have reported a convenient protocol for the synthesis 4-aryl-2-[(2*E*)-2-(3,4,5-trimethoxybenzylidene)hydrazinyl]-1,3-thiazoles and 4-aryl-2-{(2*E*)-2-[5-phenylfuran-2-yl) methylidene} hydrazinyl} -1,3-thiazoles in good yield. The antioxidant properties of the new compounds were evaluated using DPPH radical scavenging assay .Compounds **2a, 2b, 4a, 4c, 4f, 4g** and **4h** were identified as potent antioxidants. The high activity in **2b** may be attributed to the presence of –OH group in the molecule. Antioxidant activities of these compounds against the stable free radical DPPH showed that these species could help in increasing the overall antioxidant capacity of an organism. However, further detailed study on activity and long term toxicity need to be carried out before any final conclusion can be drawn.

The antibacterial and antifungal screening data of the newly synthesized compounds revealed that the most of the compounds synthesized were very active against all the bacterial and fungal strains. Compounds **2a** and **2c** showed maximum activity against all the tested microorganisms.

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