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Synthesis and Biological Evaluation of Novel 2,4-Disubstituted Thiazoles for Antimicrobial and Antioxidant Activities

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Abstract

Thiazole derivatives represent an important class for the development of new drugs because of their various biological activities. In this study the synthesis and biological evaluation of some new 2,4-disubstituted thiazoles obtained from 3-aryl-1H-pyrazole-4-carbaldehyde thiosemicarbazones and substituted phenacyl bromides are reported. The main purpose was to synthesize new thiazole derivatives with improved antimicrobial and antioxidant activity. Fourteen compounds (P25-P38) were synthesized by a typical reflux approach and its spectroscopic analyses viz., FT-IR, NMR, and mass spectrometry were confirmed. Antimicrobial activity was screened against different bacterial and fungal strains by well-plate method and antioxidant ability was evaluated by DPPH, nitric oxide, hydroxyl radical and superoxide anion scavenging methods. compounds P29 and P38 showing the best antimicrobial activities against all tested strains equivalent to streptomycin and fluconazole. Moreover, the majority of the synthetic derivatives revealed good antioxidant activities (DPPH scavenging efficacy >80%). Acute toxicity studies showed that the compounds were safe even upto 2000 mg/kg. Structure-activity relationship study revealed that the 2, 4-dichloro substitution on the pyrazole ring benefited the activity. The results indicate that new 2,4-disubstituted thiazoles may be considered as promising scaffolds for designing new antimicrobial and antioxidant agents with low toxicity.

Keywords: Thiazide derivatives, Antibacterial activity, Anti-oxidant agency, Pyrazole compounds, Structure-activity relationship.

1. Introduction

Heterocyclic nitrogen-and sulfur-containing compounds have also aroused wide interest in the medicinal chemistry area on account of the promising pharmacological properties and therapeutic applications. Of those, derivatives of thiazole have been recognized as privileged scaffolds which played an important role in the pharmaceutical field due to their diverse and potential biological activities such as antioxidant, anti-inflammatory, antimicrobial, antituberculotic and anticancer activities (Solanki & Bhatt, 2025). The thiazole ring system (5-membered heterocyclic ring system with Nitrogen and Sulfur) is an important pharmacophore in several clinically approved drugs, including riluzole, dasatinib, and meloxicam. Chemically, thiazole is a ring-shaped molecule of the five-atom ring with the formula C_3H_3NS of the atomic numbers of three carbons, one sulphur, and one nitrogen. Thiazole has a smell reminiscent of pyridine and is a flammable liquid that is clear to pale yellow in color. Its thiazole ring is found as part of thiamine and epothilone. Thiazoles frequently exist in novel and diverse natural product structures displaying multiple biological activities. The presence of thiazole containing compounds in peptides, ability in binding with proteins, DNA and RNA and antitumor, antiviral and antibiotic activities has led to many synthetic studies and new applications.

Due to the spread of drug-resistant microbial infections and oxidative stress-induced diseases, there is an urgent demand for the creation of new therapeutic products possessing superior specific action and safety. Note that nitrogen-containing heterocyclic rings have received a great deal of attention in the context of resolution of drug-resistant infections leading to new vistas in an antimicrobial regime (Li et al., 2025). The dichotomy of thiazole and pyrazole moieties linked within a single molecular scaffold is an excellent method to improve drug design as both reported heterocycles are well known for their remarkable biological effects.

2. Literature Review

Nitrogen-contained heterocyclic compounds the therapeutic potential of nitrogen-containing heterocyclic compounds has been well documented in current literature. Several synthetic methods for thiazole formation have been established. One of the oldest methods of preparation of thiazoles is the Hantzsch thiazole synthesis through the reaction of haloketones and thioamides (Sheldrake et al. 2006). In the reaction, for instance, for the dehalogenation reaction simple thiazoles were formed in good yield but other examples of the modified thiazoles were formed in the obtained in low yield because of strong nucleophilicity of the Sulphur atom in the thioamides and thioureas. Potewar et al. (2007) reported an ionic liquid with 2,4-disubstituted thiazoles. The authors emphasized that the reaction medium was a unique reaction medium with easy recovery and reuse, environment friendly (no use of toxic organic solvents, mild condition in reactions, high product yields, accelerated reaction rates, non-toxic nature of catalyst and less trash). But with the same method a practical synthesis was developed of Fanetizole, an anti-inflammatory.

Bharti et al. 2,4-disubstituted thiazole ring Schiff base were synthesized the and screened the compounds for antibacterial and antifungal activity. Many of the chemicals produced were antibacterial and antifungal. By employing a synthetic approach, Vijesh et al. (2010) reported antibacterial activities of 2,4-disubstituted thiazoles. The antimicrobial activity of the agents were good against all the tested microorganisms in compounds carrying 2,5-dichlorothiophene and 2,4-dichlorophenyl moiety. Dawane et al (2010) have reported a greener, cleaner and rapid method for the synthesis of 1,4- (4-chlorophenyl)-2-thiazolyl)-3-aryl-5-(2-butyl-4-chloro-1H-imidazol-5yl). Two, chalcones and hydrazinothiazole act together in PEG-400. to form -2-pyrazolines. The majority of tested compounds were indeed active in the present study. Liaras et al., (2011) has synthesized some thiazole chalcones and evaluated them for their antibacterial activity and found them to be potent antimicrobials. Antimicrobial investigation exhibited that the majority of these compounds were more active than reference drugs. Chimenti et al. (2011) have recently developed and screened novel anti-Candida spp. compounds consisting of 2,4-disubstituted-1,3-thiazoles. Hydrazone derivatives containing heterocyclic or bicyclic rings demonstrated good selective inhibitory activity, in particular, towards Candida albicans and Candida glabrata, the study noted. Gaikwad and coworkers 2012 prepared a series of compounds with thiazole moiety using a novel benzotriazole combination for their antibacterial activity. Microorganisms inhibitions were moderate to good for the great majority of the antimicrobial screening agents.

3. Objectives

1. To describe the methods and pyrazolone bases, including chalcones and Schiff bases for the synthesis of novel heterocyclic compounds.

2. To synthesize and evaluate novel 2,4-disubstituted thiazoles for antibacterial and antioxidant activity through comprehensive biological screening.

4. Methodology

It is performed as an experimental laboratory research in which new 2,4-disubstituted thiazole derivatives were generated and tested for their biological evaluation. The study was approved by the Institutional Ethical Committee, Kuvempu University, Shimoga, India. Experimental The synthetic procedure comprised the synthesis of 3-aryl-1H-pyrazole-4-carbaldehyde thiosemicarbazones (8a-e) via multi-steps involving condensation processes. The reaction was performed for 2 h, and refluxed under sulphuric acid in water catalytically concentrated: 10 ml of absolute ethanol containing 0.005 mol of 3-aryl-1H-pyrazole-4-carbaldehyde (3a-e) and 0.005 mol of thiosemicarbazide. The resulting solid was filtered, washed with ethanol dried and recrystallised. Substituted phenacyl bromides (9a-c) were prepared according to the Furniss et al. 1996 method. In a further funnel-dropping dry three-necked-flask, 30 cc of anhydrous ether and 0.04 mol of the substituted acetophenone were put. Remo've it from the cold bath, and mix in with constant stirring 01 gramme of anhydrous AlCl3 and 64 g (0*04 mol) of bromine by means of a spoon. On completion of the addition of all the bromine, the ether and the dissolved HBr were removed by suction.

The title compounds P25–P38 were prepared as follows: mixture of the appropriate substituted phenacyl bromides 9a–c and 8 was refluxed with absolute ethanol for 4 h. a-e, a 3-aryl-1H-pyrazole-4-carbaldehyde thiosemicarbazone (0.001 mol). After the reaction, the mixture was cooled. The filtered pure compound was recrystallized in ethanol-dioxane. The prepared compounds were characterized by the usual spectral methods. Melting points were determined by open capillary analysis. Infrared spectra were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. Chemical shift values are reported in parts per million. Mass spectra were recorded on Agilent 1100 series LC-MS. The antimicrobial activity was assayed by the "well-plate" method in Mueller-Hinton Agar. Ago all fungi were maintained on a potato dextrose agar (PDA) at ± 25 ˚C. Antioxidant evaluation the antioxidant activity was determined by several assays which includes DPPH radical scavenging, nitric oxide scavenging, hydroxyl radical scavenging, superoxide anion scavenging activities using BHT as a standard. Acute toxicity study was performed on Swiss albino mice (20-25 g) according to OECD guidelines No. 420 where the mice were administered with doses of 250-5000 mg/kg and observed for changes in behavior and mortality.

5. Results

This synthetic route led to the formation of fourteen new 2,4-disubstituted thiazole derivatives (P25-P38) with good yields. The synthesized compounds P25–38 were characterized by NMR, IR, and elemental analysis. Spectra and analytical data of all the compounds prepared corresponded to the models.

Table 1: Information on the Chemicals' Characteristics P25-38

Compounds	R	Ar	Molecular Formula (Mol. wt.)	Yield (%)	M.p. (°C)
$P_{(25)}$	H	C_6H_5	$C_{19}H_{15}N_3S$ (345)	86	209–210
$P_{(26)}$	4-OCH_3	C_6H_5	$C_{20}H_{17}N_3OS$ (375)	72	220–221
$P_{(27)}$	4-F	C_6H_5	$C_{19}H_{14}FN_3S$ (363)	88	125–127

$P_{(28)}$	4-Cl	C_6H_5	$C_{19}H_{14}ClN_3S$ (379)	74	150–152
$P_{(29)}$	2,4-Cl	C_6H_5	$C_{19}H_{13}Cl_2N_3S$ (414)	87	180–183
$P_{(30)}$	H	4-OCH_3–C_6H_4	$C_{20}H_{17}N_3OS$ (375)	89	229–231
$P_{(31)}$	4-OCH_3	4-OCH_3–C_6H_4	$C_{21}H_{19}N_3O_2S$ (405)	85	239–241
$P_{(32)}$	4-F	4-OCH_3–C_6H_4	$C_{20}H_{16}FN_3OS$ (393)	87	240–242
$P_{(33)}$	4-Cl	4-OCH_3–C_6H_4	$C_{20}H_{16}ClN_3OS$ (409)	82	243–245
$P_{(34)}$	H	4-F–C_6H_4	$C_{19}H_{14}FN_3S$ (363)	84	229–231
$P_{(35)}$	4-OCH_3	4-F–C_6H_4	$C_{20}H_{16}FN_3OS$ (393)	88	241–243
$P_{(36)}$	4-F	4-F–C_6H_4	$C_{19}H_{13}F_2N_3S$ (381)	85	258–260
$P_{(37)}$	4-Cl	4-F–C_6H_4	$C_{19}H_{13}ClFN_3S$ (397)	88	252–254
$P_{(38)}$	2,4-Cl	4-F–C_6H_4	$C_{19}H_{12}Cl_2FN_3S$ (432)	72	232–233

A few of the derivatives investigated, (14) R and Ar substituents Table 1 lists some 14 derivatives of the thiazole that have various R and Ar substituents. The yields are between 72% and 89%, P_{30} giving the highest yield and P_{26} and P_{38} from solar cells the lowest. The melting points largely range from 125–127°C (P_{27}) up to 258–260°C (P_{36}) depending on the functional group polarity and the molecular size. The molecular weights vary from 345 to 432 according to the complexity of the substitution. Thermally more stable results are usually obtained by using EWG such as F and Cl. The data shown in Table 1 indicate that substitution in the chemical leads to strong effects on yield, melting point and structure.

Table 2: The Antibacterial Properties of Certain Substances P25-38

Compound	E. coli		S. aureus		P. aeruginosa	
	1000 µg/mL	500 µg/mL	1000 µg/mL	500 µg/mL	1000 µg/mL	500 µg/mL
P_{25}	07±0.2	05±0.2	08±0.2	06±0.2	05±0.2	03±0.2
P_{26}	06±0.1	02±0.2	09±0.2	07±0.2	08±0.2	06±0.1
P_{27}	09±0.2	06±0.2	08±0.1	04±0.2	07±0.2	05±0.2
P_{28}	06±0.2	04±0.1	05±0.1	05±0.1	06±0.2	04±0.2
P_{29}	13±0.2	10±0.2	14±0.1	12±0.1	16±0.1	13±0.1
P_{30}	10±0.1	07±0.1	11±0.2	08±0.2	10±0.1	08±0.1
P_{31}	11±0.2	09±0.2	09±0.2	07±0.1	10±0.2	07±0.2
P_{32}	0	0	0	0	0	0
P_{33}	10±0.2	07±0.2	10±0.1	08±0.1	07±0.2	03±0.2
P_{34}	13±0.2	10±0.1	09±0.2	07±0.1	10±0.2	08±0.1
P_{35}	0	0	0	0	0	0
P_{36}	10±0.2	08±0.2	08±0.2	06±0.2	09±0.2	07±0.1
P_{37}	09±0.2	07±0.1	11±0.2	09±0.2	10±0.2	08±0.1

P_{38}	15±0.2	12±0.2	12±0.1	11±0.2	16±0.2	13±0.2
Streptomycin (Std.)	16±0.2	10±0.1	15±0.2	10±0.2	16±0.2	12±0.1

In Table 2 the data about antibacterial activity (zone of inhibition) of compounds P_{25}–P_{38} versus E. coli, S. aureus, and P. aeruginosa at 1000 µg/mL and 500 µg/mL are presented. P_{38} proved the most potent activities toward all strains, with zones that ranged up to 16±0.2 mm approximating the standard streptomycin. In addition, P 29, P 34, and P 37 had significant inhibitory activities. Neither P_{32} nor P_{35} were active at either concentration. Activity generally increased with concentration. In general, halogenated derivatives were more active, and P_{38} was considered the most promising compound among the tested derivatives.

Table 3: Antifungal Activity of Compounds P25-38

	A. flavus		**C. keratinophilum**		**C. albicans**	
Compound	**1000 µg/mL**	**500 µg/mL**	**1000 µg/mL**	**500 µg/mL**	**1000 µg/mL**	**500 µg/mL**
P_{25}	04±0.2	02±0.2	06±0.2	05±0.2	05±0.2	03±0.2
P_{26}	05±0.1	03±0.1	04±0.2	03±0.1	03±0.1	1
P_{27}	06±0.1	05±0.2	06±0.1	03±0.1	05±0.1	04±0.1
P_{28}	04±0.2	04±0.1	03±0.1	03±0.1	04±0.2	03±0.1
P_{29}	11±0.1	09±0.1	09±0.1	07±0.1	10±0.2	08±0.2
P_{30}	0	0	0	0	0	0
P_{31}	03±0.1	1	02±0.1	1	03±0.1	1
P_{32}	0	0	0	0	0	0
P_{33}	0	0	0	0	0	0
P_{34}	04±0.1	1	03±0.1	1	04±0.1	02±0.1
P_{35}	0	0	0	0	0	0
P_{36}	06±0.2	05±0.1	04±0.2	03±0.1	05±0.1	03±0.1
P_{37}	04±0.2	03±0.1	03±0.2	2	04±0.2	2
P_{38}	10±0.1	08±0.1	09±0.1	07±0.1	11±0.1	08±0.1
Fluconazole (Std.)	13±0.2	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2

Table 3 Antifungal activity of P_{25} –P_{38} against Aspergillus flavus, Chrysosporium keratinophilum and Candida albicans. These were P_{29} and P_{38} with pronounced activity against all three fungi and inhibition zones from 10–11 mm (P_{38}), which are the nearest to the standard (fluconazole, 22 mm against Candida albicans). P_{30}, P_{32}, P_{33}, and P_{35} were inactive. At 1000 µg/mL, antifungal activity was enhanced and concentration-dependent inhibition was observed. The results in Table 3 suggest that the introduction of a halogen X (Cl, F) atom favours antifungal properties and the derivative P38 is the most potent.

Table 4: Scavenging Activity (%) of Compounds P25-38

Compound	DPPH Assay	Nitric Oxide Assay	OH Radical Assay	Superoxide Anion Scavenging Activity
P_{25}	80.88	60.46	60.46	66.46
P_{26}	86.69	77.49	77.49	82.49
P_{27}	68.22	58.22	58.22	62.22
P_{28}	70	60.24	60.24	64.24
P_{29}	74.55	64.56	64.56	62.32
P_{30}	76.62	76.62	76.62	68.56
P_{31}	83.06	61.46	61.46	63.24
P_{32}	82.56	62.16	62.16	65.16
P_{33}	84.47	64.42	64.42	60.64
P_{34}	86.08	72.24	72.24	76.24
P_{35}	84.1	64.1	64.1	68.46
P_{36}	85.11	66.11	66.11	66.87
P_{37}	84.74	62.56	62.56	64.26
P_{38}	86.18	74.34	74.34	78.56
BHT	92.42	88.56	89.56	85.86

The antioxidant scavenging activity of compounds P_{25}–P_{38} are shown in Table 4 at DPPH, nitric oxide, OH radical, and superoxide assays. The P2 6, P3 4, and P3 8 displayed high activity, in particular the P2 6 by 86.69% DPPH and 82.49% superoxide scavenging. P_{27} and P_{28} exhibited lowest activity in all assays. The conventional BHT was highest in all the assays ranging from 92.42% (DPPH). Most of methoxy or halogen substituted compounds worked better. Therefore, Table 4 indicates that substitution has a strong influence on the antioxidant potential, because molecules such as P_{26} and P_{38} are near standard efficacy.

Table 5: Acute Toxicity Assessment

Dose (mg/kg)	P29	P38
	Mortality/Behavior	Mortality/Behavior
250	0/6 Normal	0/6 Normal
500	0/6 Normal	0/6 Normal
1000	0/6 Normal	0/6 Normal
2000	0/6 Normal	0/6 Normal
3000	1/6 Depression (Day 1)	1/6 Depression (Day 1)
4000	6/6 Death (Day 2)	6/6 Death (Day 2)
5000	6/6 Death (Day 2)	6/6 Death (Day 2)

Table 5 summarizes the toxicity assay of the most active compounds P29 and P38 according to OECD guidelines No. 420. Acute oral toxicity of P29 and P38 was investigated. (Ujihashi et al_1994); A study performed reported 2000

mg/kg to be the nontoxic dose for the compounds in rats. There were no remarkable behavioral changes observed in the test animals except for three and four thousand milligrammes per kilogramme for all the chemicals which appeared to be in a state of depression on day one and died on the following day. Both complexes showed excellent safety at doses of up to 2000 mg /kg, and no mortality has been recorded, as no significant changes in behavior also have been noticed in the treated animals. The LD50 for both compounds were estimated to be in the region of 2000-3000 mg/kg indicating moderate acute toxicity and indicating them as promising for clinical development.

6. Discussion

The prepared 2,4-disubstituted thiazole derivatives (P_{25}–P_{38}) showed good chemical, biological and antioxidant activities as can be seen from Tables 1–5. Structurally, these compounds were highly pure with consistent analytical data (NMR, IR, and elemental analysis). The successful synthesis was proved by Table 1 with good yields (72–89%) with 125–260°C as melting points indicating their stable crystalline nature. Hydrogen substituted was also investigated, specifically when halogen substituted species (P_{36}, P_{38}) were compared to P_{30} and P_{32} they were found to have larger melting temperatures, indicating an improved thermal stability. The antibacterial effects displayed in Table 2 indicated that the inhibition zones of compound P_{38} among E. coli, S. aureus and P. aeruginosa were the largest which was similar to the positive drug, streptomycin. $P_{29}$and P_{34} exhibited strong antibacterial activity, but P_{32} and P_{35} were devoid of activity, notes suggesting the presence of a halogen at ortho or para positions as the aryl group substitution could improve the antimicrobial activity. This observation was also confirmed through antifungal activity trend in Table 3, P_{29} and P_{38} seemed to have better rampant activity against A.albicans and A.flavus which was at par to fluconazole. On the other hand, compounds without halogen/met- hoxy substituents clearly didn't present significant antifungal activity.

The antioxidant activity shown in Table 4 is another proof of the activity of methoxy and halogen substitutions. P_{26}, P_{34} and P_{38} demonstrated excellent radical scavenging in all the assays which were close to the BHT standard. Strong structure–activity relationships are implied, as they maintained significant activity in several biological assays. By acute toxicity of Table 5, it can be seen that both P2 9 and P3 8 were safe up to 2000mg/kg except mild behavioral changes and deaths were observed above them. This provides a comfortable therapeutic window and an excellent basis for subsequent in vivo and clinical studies. Consistently, these results identify P38 as the most attractive multifunctional lead compound.

7. Conclusion

The current study has successfully executed the synthesis and in-depth biological evaluation of fourteen newly designed 2,4 disubstituted thiazole compounds (P_{25}–P_{38}) that were developed from pyrazolone based thiosemicarbazones and substituted phenacyl bromides. Confirmation of the identity and purity of these synthesized compounds was performed by NMR, IR, and HRMS and the yield, melting point, and molecular stability of these compounds(customers) are presented in Table 1, which indicates the effects of substituents. Rimnach et al 40 reported that the halogen derivatives, especially the fluorinated and chlorinated ones, had better thermal and chemical stability. The biological studies showed a good antibacterial activity of the compounds P_{29}, P_{34} and particularly P_{38}, which showed a broad spectrum inhibition close to the standard streptomycin (data is shown in Table 2). Moreover,

antifungal tests (Table 3) supported this tendency, where P_{29} and P_{38} were further great inhibitors of A. flavus, C. keratinophilum and C. albicans, being in the vicinity to the one of fluconazole. These results are highlighting the halogen groups to enhance the antimicrobial activity. Compounds P_{26}, P_{34} and P_{38} showed strong free radical scavenging potential (Table 4) with compound P_{26} possessing DPPH inhibition and superoxide anion scavenging potential of at least 86.69% and 82.49% respectively—comparable to the corresponding standard BHT. The structure–activity relationship revealed that methoxy and halogen are the most critical functionalities for potent bioactivity which were consistently demonstrated. The toxicological studies (Table 5) revealed the non-toxic nature of P_{29} and P_{38} up to 2000 mg/kg and its LD_{50} value was calculated around 2000–3000 mg/kg, suggested as moderate toxicity and good therapeutic candidate. Overall, compound P_{38} turned out to be the most promising lead, because of its potent and broad spectrum antimicrobial, antioxidant, and safety profiles. These results provide support for its continued development as a potential anticancer therapy and emphasizes the value of judicious substitutions in the design of heterocyclic drugs.

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