Synthesis and Antibacterial Activity of 2-(2,4-Dinitrophenyl)-3,5-diphenyl (substituted)-6-aryl-3,3a,5,6-tetrahydro-2H-pyrazolo[3,4-d] thiazoles

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Condensation of substituted benzaldehydes with primary aryl amines gave a series of Schiff bases($1a_1$-e_1,a_2,b_2,d_2,b_3-e_3) which, on reaction with thioglycolic acid, resulted in the formation of the corresponding 4-thiazolidinones($2a_1$-e_1,a_2,b_2,d_2,b_3-e_3). These compounds, on condensation with substituted benzaldehydes in anhydrous sodium acetate, furnished 2-phenyl(substituted)-3-aryl-5-benzilidine(substituted)-thiazolidine-4-ones($3a_1$-e_1,a_2,b_2,d_2, b_3-e_3). The latter, on heating with 2,4-dinitrophenyl hydrazine in anhydrous sodium acetate, gave the title compounds($4a_1$-e_1,a_2,b_2,d_2,b_3-e_3). The structures have been established on the basis of elemental analysis and spectral data. The title compounds have been screened *in vitro* for their possible antibacterial activity.

Selected substituted thiazoles[1-3] as well as different heterocyclic systems containing pyrazole ring[4-6] possess potent biological activities. It is also believed that the presence of N-C-S linkage is responsible for the amoebicidal, anticonvulsant, fungicidal[7], and antiviral activities[8]. The present investigation deals with the development of a new series of nitrogen heterocyclic systems from easily available starting materials.

Substituted benzaldehydes on condensation with primary aryl amines gave Schiff bases ($1a_1$-e_1,a_2,b_2,d_2,b_3-e_3) which, on reaction with thioglycolic acid in refluxing benzene, furnished the corresponding 4-thiazolidinones($2a_1$-e_1,a_2,b_2,d_2,b_3-e_3). The latter, on condensation with substituted benzaldehydes in the presence of anhydrous sodium acetate and glacial acetic acid, afforded the formation of 2-phenyl(substituted)-3-aryl-5-benzilidine (substituted)-thiazolidine-4-ones($3a_1$-e_1,a_2,b_2,d_2,b_3-e_3) which, in turn, heated with 2,4-dinitrophenyl hydrazine in the presence of anhydrous sodium acetate and glacial acetic acid, furnished the bridgehead nitrogen heterocyclic system, 2-(2,4-dinitrophenyl)-3,5-diphenyl(substituted)-6-aryl-3,3a,5,6-tetrahydro-2H-pyrazolo[3,4-d]thiazoles($4a_1$-e_1, a_2,b_2,d_2,b_3-e_3) (Scheme 1). Newly synthesised compounds were characterised by IR, NMR spectral data and elemental analysis. The title compounds were evaluated for their possible antibacterial activity.

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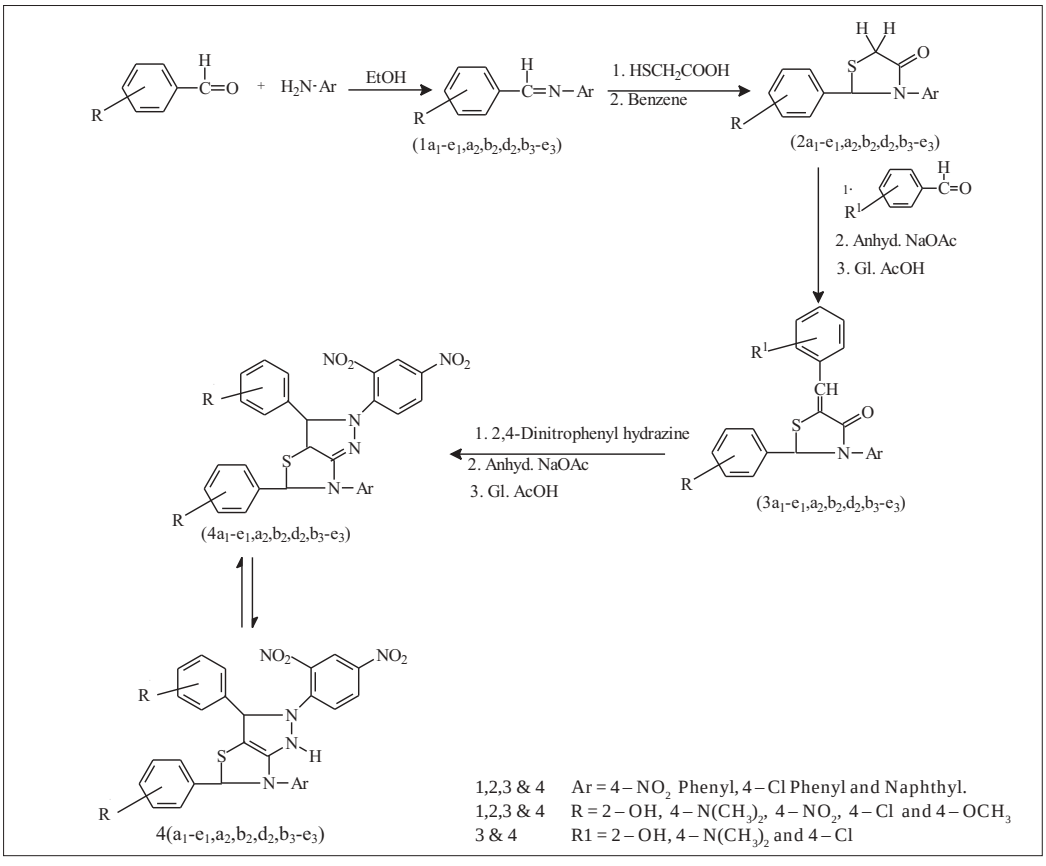
Melting points were determined in open capillaries and were uncorrected. Purity of the compounds was checked by TLC. IR spectra were recorded on a Jasco FT/IR 410 spectrophotometer in KBr disc. ^{1}H NMR spectra were taken on a Bruker DPX, 300 MHz, spectrometer using TMS as internal reference. C, H, and N analyses were carried out on a Euro EA analyzer (Italy). The bacteria used in the antibacterial activity study were procured from the Department of Bacteriology and Virology, Faculty of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar.

Schiff bases($1a_1$-e_1,a_2,b_2,d_2,b_3-e_3) and the corresponding 4-thiazolidinones($2a_1$-e_1, a_2,b_2,d_2,b_3-e_3) were synthesised following the reported method[9].

Synthesis of 2-phenyl (substituted)-3-aryl-5-benzilidine (substituted)-thiazolidine-4-ones($3a_1$-e_1,a_2,b_2,d_2,b_3-e_3) was achieved by refluxing an equimolar (0.001 mol) mixture of 4-thiazolidinone derivatives ($2a_1$-e_1,a_2,b_2,d_2,b_3-e_3), substituted benzaldehydes, and anhydrous sodium acetate (0.082 g) in glacial acetic acid (20 ml) for 3 h. The reaction mixture was concentrated, cooled, and poured into ice-cold water. The solid thus separated was filtered, washed with water, dried, and recrystallised from glacial acetic acid. The yield and melting points are given in



Scheme 1: Synthetic route.

Table 1. ($3c_1$): IR(KBr, v_{max} in cm^{-1}): 3290(OH), 3045(CH-arom.),1736(C=O), 1538(asym.NO_2), 1337 (sym.NO_2). ($3a_2$): IR(KBr, v_{max} in cm^{-1}): 3286(OH), 3026(CH-arom.), 1722(C=O), 754(C-Cl). ($3b_3$): IR (KBr, v_{max} in cm^{-1}): 3063(CH-arom.), 1742(C=O), 743(C-Cl). The NMR spectra of the synthesised compounds (3) of the series revealed peaks around 5.1-5.8 δ (s, 1H, C=CH) and 6.5-8.0 δ due to bulk aromatic protons.

Synthesis of 2-(2,4-dinitrophenyl)-3,5-diphenyl(substituted)-6-aryl-3,3a,5,6-tetrahydro-2H-pyrazolo[3,4-d]thiazoles($4a_1$-e_1,a_2,b_2,d_2,b_3-e_3) was performed by heating an equimolar (0.001 mol) mixture of 2-phenyl(substituted)-3-aryl-5-benzilidine(substituted)-thiazolidine-4-ones ($3a_1$-e_1,a_2,b_2,d_2, b_3-e_3), 2,4-dinitrophenyl hydrazine (0.198 g) and anhydrous sodium acetate (0.082 g) in glacial acetic acid (20 ml) under reflux for 6 h, which was then cooled to room temperature. The solid thus separated was filtered, washed thoroughly with water, dried, and recrystallised from glacial acetic acid. The yield and melting points are given in Table 2. ($4c_1$):IR(KBr, v_{max} in cm^{-1}): 3294(OH), 3031(CH-arom.), 1617(C=N), 1520(asym.NO_2), 1346

TABLE 1: PHYSICAL DATA OF THE SYNTHESISED COMPOUNDS ($3a_1$-e_1,a_2,b_2,d_2,b_3-e_3)

Compd.	Substituents			mp (°)	Yield (%)	Molecular formula[a]
	Ar	R	R^1			
$3a_1$	4-NO_2Ph	2-OH	2-OH	152	47	$C_{22}H_{16}N_2O_5S$
$3b_1$	4-NO_2Ph	4-$N(CH_3)_2$	2-OH	88	45	$C_{24}H_{21}N_3O_4S$
$3c_1$	4-NO_2Ph	4-NO_2	2-OH	132	44	$C_{22}H_{15}N_3O_6S$
$3d_1$	4-NO_2Ph	4-Cl	2-OH	202	42	$C_{22}H_{15}N_2O_4SCl$
$3e_1$	4-NO_2Ph	4-OCH_3	2-OH	190	46	$C_{23}H_{18}N_2O_5S$
$3a_2$	4-Cl Ph	2-OH	4-$N(CH_3)_2$	108	48	$C_{24}H_{21}N_2O_2SCl$
$3b_2$	4-Cl Ph	4-$N(CH_3)_2$	4-$N(CH_3)_2$	86	58	$C_{26}H_{26}N_3OSCl$
$3d_2$	4-Cl Ph	4-Cl	4-$N(CH_3)_2$	118	48	$C_{24}H_{20}N_2OSCl_2$
$3b_3$	Naphthyl	4-$N(CH_3)_2$	4-Cl	206	64	$C_{28}H_{23}N_2OSCl$
$3c_3$	Naphthyl	4-NO_2	4-Cl	98	65	$C_{26}H_{17}N_2O_3SCl$
$3d_3$	Naphthyl	4-Cl	4-Cl	80	49	$C_{26}H_{17}NOSCl_2$
$3e_3$	Naphthyl	4-OCH_3	4-Cl	95	69	$C_{27}H_{20}NO_2SCl$

[a]All the compounds showed satisfactory C, H, and N analysis

TABLE 2: PHYSICAL DATA OF THE SYNTHESISED COMPOUNDS ($4a_1$-e_1, a_2, b_2, d_2, b_3-e_3)

Compd.	Substituents			mp (^{0})	Yield (%)	Molecular formula[b]
	Ar	R	R^1			
$4a_1$	4-NO_2Ph	2-OH	2-OH	238	52	$C_{28}H_{20}N_6O_8S$
$4b_1$	4-NO_2Ph	4-$N(CH_3)_2$	2-OH	216	54	$C_{30}H_{25}N_7O_7S$
$4c_1$	4-NO_2Ph	4-NO_2	2-OH	236	68	$C_{28}H_{19}N_7O_9S$
$4d_1$	4-NO_2Ph	4-Cl	2-OH	222	65	$C_{28}H_{19}N_6O_7SCl$
$4e_1$	4-NO_2Ph	4-OCH_3	2-OH	196	62	$C_{29}H_{22}N_6O_8S$
$4a_2$	4-Cl Ph	2-OH	4-$N(CH_3)_2$	180	60	$C_{30}H_{24}N_6O_5SCl$
$4b_2$	4-Cl Ph	4-$N(CH_3)_2$	4-$N(CH_3)_2$	200	42	$C_{32}H_{30}N_7O_4SCl$
$4d_2$	4-Cl Ph	4-Cl	4-$N(CH_3)_2$	138	68	$C_{30}H_{24}N_6O_4SCl_2$
$4b_3$	Naphthyl	4-$N(CH_3)_2$	4-Cl	160	64	$C_{34}H_{27}N_6O_4SCl$
$4c_3$	Naphthyl	4-NO_2	4-Cl	244	63	$C_{32}H_{21}N_6O_6SCl$
$4d_3$	Naphthyl	4-Cl	4-Cl	199	61	$C_{32}H_{21}N_5O_4SCl_2$
$4e_3$	Naphthyl	4-OCH_3	4-Cl	220	65	$C_{33}H_{24}N_5O_5SCl$

[b]All the compounds showed satisfactory C, H, and N analysis

TABLE 3: ANTIBACTERIAL ACTIVITY DATA OF THE SYNTHESISED COMPOUNDS ($4a_1$-e_1, a_2, b_2, d_2, b_3-e_3)

Compd.	Zone of inhibition (mm*)			
	S.a.	A.p.	E.c.	K.a.
$4a_1$	17	15	16	18
$4b_1$	16	19	18	17
$4c_1$	18	16	15	19
$4d_1$	21	18	19	20
$4e_1$	16	15	17	18
$4a_2$	19	20	21	17
$4b_2$	22	21	20	21
$4d_2$	21	22	23	22
$4b_3$	21	20	22	23
$4c_3$	20	22	23	24
$4d_3$	22	23	24	22
$4e_3$	20	18	19	17
Ampicilin trihydrate	29	30	32	31
DMSO	00	00	00	00

*Diameter of cup is 6 mm. S.a.-*Staphylococcus aureus*, A.p.-*Actinomycus pyoginus*, E.c.-*Escherichia coli*, K.a.-*Klebsiella aeruginosa*

(sym.NO_2); ^{1}H NMR($CDCL_3$) δ ppm: 3.18(s,1H,CH), 5.82 (s, 1H, CH), 6.60-8.75 (m, 15H, Ar-H), 11.20 (s, 1H, OH). ($4a_2$): IR (KBr, ν_{max} in cm^{-1}): 3285 (OH), 3078 (CH-arom.), 1611 (C=N), 1539(asym.NO_2), 1358 (sym.NO_2), 751 (C-Cl); ^{1}H NMR ($CDCl_3$) δ ppm: 2.02 (s, 6H, 2×CH_3), 3.16 (s, 1H, CH), 5.75 (s, 1H, CH), 5.90 – 7.97(m, 15H, Ar-H), 10.99 (s, 1H, OH). ($4b_3$): IR(KBr, ν_{max} in cm^{-1}): 3059 (CH-arom.), 1614 (C=N), 1518 (asym. NO_2), 1337 (sym. NO_2), 756 (C-Cl); ^{1} H NMR ($CDCl_3$) δ ppm: 1.98 (s, 6H, 2×CH_3), 3.22 (s, 1H, CH), 5.83 (s, 1H, CH), 6.39- 8.88 (m, 18H, Ar – H).

The IR spectra of the title compounds exhibited prominent peaks around 3080-3030 cm^{-1}(CH-arom), 1620-1600 cm^{-1}(C=N), 1550-1510 cm^{-1}(asym. NO_2) and 1360-1330 cm^{-1}(sym. NO_2). The NMR spectra of the final compounds (4) of the series revealed peaks around 3.16-3.22 δ (s, 1H, CH), 5.75-5.83 δ (s, 1H, CH) and 5.90-8.88 δ due to bulk aromatic protons. No doublet was seen in the NMR spectrum of any compound of the series, thus indicating that the initial structure got rapid transformation through the tautomeric shift of H-atom to the more stable structure, as indicated in the Scheme 1.

The antibacterial activity of the title compounds was determined by agar cup-plate method[10] against *Staphylococcus aureus*, *Actinomycus pyoginus*, *Escherichia coli*, and *Klebsiella aeruginosa*. The medium was prepared as per the instructions of the manufacturer of dry Mueller Hinton agar powder (Hi-Media). The test samples were dissolved in dimethyl sulphoxide (DMSO) at a concentration of 100 μg/ml. Ampicillin trihydrate (100 μg/ml) in DMSO was used as reference standard, and the solvent control (only DMSO) was also maintained throughout the experiment. The zones of inhibition are reported in Table 3.

Synthesised compounds were screened for antibacterial activity by agar cup-plate method, the results of which revealed promising activity for most of the test compounds. Among the compounds, $4b_2$, $4d_2$, $4b_3$, $4c_3$, and $4d_3$ were found to be most active against all the microbes tested. Even though the test compounds are less active with reference to the standard drug ampicillin trihydrate, the data reported in this article may be a helpful guide for the medicinal chemists who are working in the area.

ACKNOWLEDGEMENTS

The authors wish to thank the authorities of Utkal University, Bhubaneswar, for providing laboratory facilities.

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Accepted 5 June 2006
Revised 18 July 2005
Received 21 March 2005
Indian J. Pharm. Sci., 2006, 68 (3): 377-380