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CONDUCTOMETRIC STUDY OF COMPLEX FORMATION BETWEEN 2,3-PYRAZINEDICARBOXYLIC ACID AND SOME TRANSITION METAL IONS IN METHANOL

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ABSTRACT

The complexation reactions between $CuCl_2$, $CoCl_2$ and $NiCl_2$ with 2,3-Pyrazinedicarboxylic acid in methanol (MeOH) at 313.15 K were studied by conductometric methods. The association constants, formation constants and Gibbs free energies were calculated from the conductometric titration curves. On drawing the relation between molar conductance and the ratio of metal to ligand concentrations, different lines were obtained indicating the formation of 1:1 and 2:1 (M:L) stoichiometric complexes. The formation constants and Gibbs free energies of different complexes in absolute Methanol at 313.15 K follow the order:)

$K_f(2:1)>K_f(1:1)$ *for (M:L) and* $\triangle G_f(2:1)>\triangle Gf(1:1)$ *for (M:L)*

KEY WORDS: Association constants; formation constants; Gibbs free energies of association; Gibbs free energies of complex formation.

RESUMO

A formação de complexos entre $CuCl_2$, $CoCl_2$, $NiCl_2$ e ácido 2,3-pirazinodicarboxílico em metanol á 313.15 K foi estudada usando métodos de condutividade. As constantes de associação e formação e as energias livres de Gibbs foram calculadas a partir de curvas de titulação condutimétrica. A relação entre a condutância molar e a proporção das concentrações metal-ligante levou a linhas retas indicando a formação de complexos estequiométricos (M:L) 1:1 e 2:1. As constantes de formação e as energias livres de Gibbs dos vários complexos em metanol à 313.15 K seguem a ordem:

$K_f(2:l)>K_f(1:1)$ *para (M:L) e* $\triangle Gf(2:1)>\triangle G_f(1:1)$ *para (M:L)*

PALAVRAS CHAVE: Constantes de associação. Constantes de formação,Energias livres de Gibbs de associação, energias livres de Gibbs para formação de complexos.

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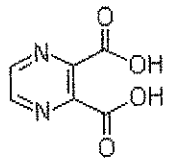
INTRODUCTION

The long range ion – ion interactions due to screened columbic forces are the most important features of electrolyte in solutions. These act together with shorter – ranged forces between the solvent molecules and between the solvent molecules and ion. Electrical conductivity (EC) is a measure measure of solvent to conduct electric current and depends on: concentration of the ions, ligand and temperature in solutions. Current is carried out by both cations and anions, but to different degree. The conductivity due to divalent cations is more than that of mono-valet cations, it is not true for anions. Metal cations with d^o noble gas electron configuration (alkali and alkaline earth) metal ions together with the inert molecular ions like tetraalkylammonium,-phosphonium,-arsonium , and trialkylsulfonium ions exhibit properties mainly determined by their charge and size [1].Solvation of such cations in protic and polar solvents is due essentially to electrostatic ion-dipole and ion induced dipole interactions. Metal cations with filled d - orbitals, the d^{10} cations, exhibit partially covalent character in their interactions; their properties depend on the charge and size and partially on their electro negativity. Cations with incomplete d- orbitals called d^n-cations .With these cations protic and polar solvent molecules are strongly bound in complexes to a central cation through p-d orbital overlap and exchange only slowly with the bulk solvent. The formation of complexes becomes more important at high concentration of the complex ion and is likely to be more extensive in non-aqueous solvents, particularly in dipolar aprotic solvents, whereas the salvation of anions is weaker, leading to stronger complexation. Therefore conductivity study is valuable on using transition metal cations [2-7]. This work provides the analytical analyst and the biological analyst data can help him for deterring the concentration of $CuCl_2$, $CoCl_2$ and $NiCl_2$ in blood and different solutions.

2,3-Pyrazinedicarboxylic acid

Identification

Name	2,3-Pyrazinedicarboxylic acid
Synonyms	Pyrazine-2,3-dicarboxylic acid
Molecular Structure	
Molecular Formula	$C_6H_4N_2O_4$
Molecular Weight	168.11
CAS Registry Number	89-01-0
EINECS	201-875-3

Properties

Melting point	185-188 °C
Water solubility	Soluble

EXPERIMENTAL

The chemicals used 2, 3-pyrazine dicarboxylic acid and methanol were provided from Merck Co. and used directly without purification.

The experimental procedure to obtain the formation constant of complexes of 2,3-Pyrazinedicarboxylic acid with $CuCl_2$, $CoCl_2$ and $NiCl_2$ by conductometric procedure was as follows : -

A solution of metal chloride ($1x10^{-3}$ M) was placed in a titration cell, at a const temperature (313.15) K , and the conductance of the solution was measured . The ligand (1×10^{-2} M) was transferred step-by-step to the titration cell using a precalibrated micropipette and the conductance of the solution was measured after each transfer. Addition of the ligand solution was continued until the total concentration of the (2, 3-Pyrazinedicarboxylic acid) was approximately four times higher than that of metal ions. The conductance of the solution was measured after each addition. The complex formation constant, K_f, and the molar conductance of the complex, ML, were evaluated by computer fitting to the molar conductance mole ratio data.

RESULTS AND DISCUSSIOIN

- The stability of a transition metal complex with a polydentate chelate ligand depends on a range of factors including: number and type of the donor atoms present, the number and size of the chelate rings formed on complexation. In addition, the stability and selectivity of complexities strongly depend on the donor ability and dielectric constant of the solvent and shape and size of the solvent molecules.

- 2, 3-Pyrazinedicarboxylic acid is a polydentate ligand which tends to be completely coordinated to a metal ion. This reagent is soluble in water and soluble in most organic solvents

- The specific conductance values (Ks) of $CuCl_2$, $CoCl_2$ and $NiCl_2$ in absolute (MeOH) were measured experimentally in absence and in the presence of ligand at 313.15 K.

The molar conductance (Λ_m) values were calculated [8] using equation (1):

$$\Lambda_m = \frac{(K_s - K_{solv})K_{cell} \times 1000}{C} \quad (1)$$

Where K_s and K_{solv} are the specific conductance of the solution and the solvent, respectively; K_{cell} is the cell constant and C is the molar concentration of the $CuCl_2$, $CoCl_2$ and $NiCl_2$ solutions.

- The limiting molar conductances (Λ_o) at infinite dilutions were estimated $CuCl_2$, $CoCl_2$ and $NiCl_2$ in absolute methanol (MeOH) alone and in the presence of the ligand by extrapolating the relation between Λ_m and $C_m^{1/2}$ to zero concentration (Fig.1). By drawing the relation between molar conductance (Λ_m) and the molar ratio of metal to ligand (M/L) concentrations , different lines are obtained with sharp breaks indicating the formation of 1:1 and 2:1 (M:L) stoichiometric complexes (Fig.2).

- The experimental data of (Λ_m) and (Λ_o) were analyzed for the determination of association and formation constants for each type of the stoichiometric complexes.

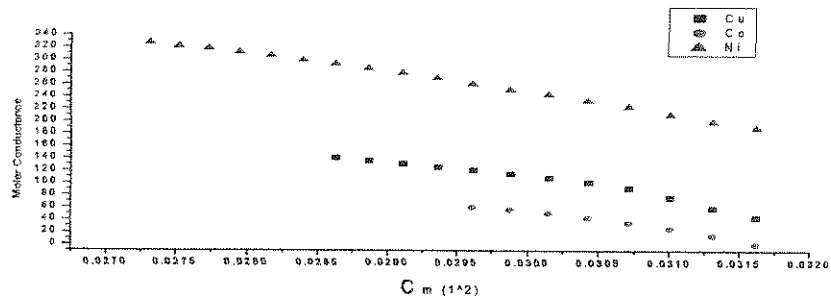


Figure 1. The relation between molar conductance (Λ_m) and ($\sqrt{C}$) of $CuCl_2$, $CoCl_2$ and $NiCl_2$ in the presence of H_2L in absolute methanol at 313.15 K.

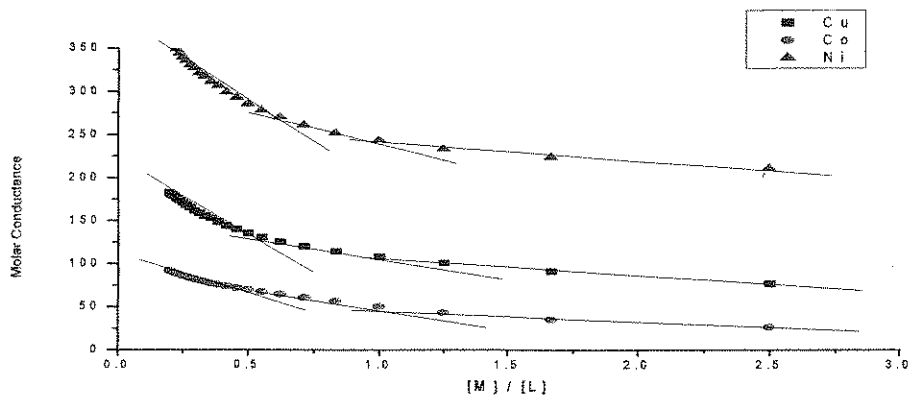


Figure 2. The relation between molar conductance (Λ_M) and the molar ratio (M/L) of $CuCl_2$, $CoCl_2$ and $NiCl_2$ in the presence of H_2L in absolute methanol at 313.15 K indicating the formation of 1:1 and 2:1 (M:L) stoichiometric complexes.

- The association constants of $CuCl_2$, $CoCl_2$ and $NiCl_2$ in the presence of ligand in absolute MeOH at 313.15 K for 1:2 asymmetric electrolytes were calculated [9, 10] by using equation (2):

$$K_A = \frac{\Lambda_0^2(\Lambda_0 - \Lambda_m)}{4C_m^2 + \Lambda^3 S(z)} \qquad (2)$$

Where (Λ_m, Λ_0) are the molar and limiting molar conductance, respectively of $CuCl_2$, $CoCl_2$ and $NiCl_2$, C_m is molar concentration of $CuCl_2$, $CoCl_2$ and $NiCl_2$, S(Z) is Fuoss-Shedlovsky factor , equal one for strong electrolytes [11]. The calculated association constants are shown in Table 1.

- The Gibbs free energies of association (ΔG_A) were calculated from the association constant [12,13] by applying equation (3) :

$$\Delta G_A = -R\,T \ln K_A \qquad (3)$$

Where R is the gas constant (8.341 J) and T is the absolute temperature (313.15 K). The calculated Gibbs free energies were presented in Table 1.

Table 1. Association constants and Gibbs free energies of association for $CuCl_2$, $CoCl_2$ and $NiCl_2$ in the presence of ligand in absolute MeOH at 313.15 K .

C	Λ_m			$\Lambda_0^2(\Lambda_0-\Lambda_m)$			$4C^2+\Lambda^3_m$			K_A			ΔG_A (k J/mol)		
	Cu	Co	Ni	Cu	Co	Ni	Cu	Co	Ni	Cu	Co	Ni	Cu	Co	Ni
0.001	44.2386	1.5347	190.1071	4153605	409822.3	35726553	7828.18	3.614689	6670605	530.622	113404.6	5.199913	-16.3877	-30.4001	-4.30623
0.00098	58.76107	14.99206	199.8977	3698561	334627.8	34109455	13820.86	3369.845	7987726	267.6072	99.30655	4.270234	-14.5997	-12.0105	-3.79174
0.000962	76.43012	26.64074	211.637	3146071	269453	32170481	23366.25	16907.72	9479266	134.6416	14.25095	3.393774	-12.8056	-6.93958	-3.19169
0.000943	91.21989	35.77829	224.4413	2683089	218328.1	30055600	33284.27	45799.27	11305987	80.6113	4.767064	2.658379	-11.4657	-4.07922	-2.55378
0.000926	100.9503	43.57271	234.2381	2378486	174717.9	28437481	40763.85	82726.31	12852051	58.34791	2.112	2.21288	-10.6214	-1.95281	-2.07445

- The association free energies evaluated for CuCl2, CoCl2 and NiCl2 -ligand complexes are small and spontaneous indicating electrostatic attraction.
- The formation constants (K_f) for $CuCl_2$, $CoCl_2$ and $NiCl_2$ complexes were calculated for each type of complexes (1:1) and (2:1) (M:L) by using equation (4) [14,15] :

$$K_f = \frac{\Lambda_M - \Lambda_{obs}}{(\Lambda_{obs} - \Lambda_{ML})[L]} \qquad (4)$$

Where Λ_m is the molar conductance of the CuCl2, CoCl2 and NiCl2 alone, Λ_{obs} is the molar conductance of solution during titration and Λ_{ML} is the molar conductance of the complex.

- The obtained values (K_f) for $CuCl_2$, $CoCl_2$ and $NiCl_2$ -ligand stoichiometric complexes are presented in Table 2, 3. The Gibbs free energies of formation for each stoichiometric complexes were calculated by using the equation :

$$\Delta G_f = -R\,T\,\ln K_f \qquad (5)$$

- The calculated ΔG_f values are presented in Tables 2, 3.

Table 2. Formation constants and Gibbs free energies of formation for 1: 1 (M/L) , $CuCl_2$, $CoCl_2$ and $NiCl_2$-H_2L in absolute MeOH at 313.15 K .

[L]	Λ_{obs}			$(\Lambda_{obs}-\Lambda_{ML})/[L]$			$(\Lambda_m-\Lambda_{obs})$			K_f			ΔG_f (k J/mol)		
	Cu	Co	Ni	Cu	Co	Ni	Cu	Co	Ni	Cu	Co	Ni	Cu	Co	Ni
0.001925	130.5947	70.41452	279.0194	0.03436	0.026712	0.054091	361.4053	422.3387	170.9806	11100.35	15810.72	3161.006	-24.3299	-25.2538	-21.0491
0.001379	125.2748	67.68132	270.7058	0.023731	0.020429	0.037443	366.7252	425.0387	179.2942	16296.39	20805.3	4788.516	-25.3328	-25.9709	-22.1339
0.001228	120.062	64.96128	261.3417	0.014727	0.013209	0.021637	391.9381	429.0942	188.6583	26513.65	32485.43	8639.321	-26.514	-27.1347	-23.6752
0.001071	114.3794	60.90575	252.1424	0.00676	0.007413	0.009195	397.6206	432.9314	197.8576	58818.62	58403.4	21517.08	-28.6654	-28.6669	-26.0567
0.000909	108.2582	57.08659	244.2408	0.000171	0.000831	0.000519	403.7419	438.9357	205.7592	2360436	528080.5	332449.7	-36.3292	-34.4181	-33.2094

Table 3. Formation constants and Gibbs free energies of formation for 2:1 (M/L) $CuCl_2$, $CoCl_2$ and $NiCl_2$-H_2L in absolute MeOH at 313.15 K.

[L]	Λ_{obs}			$(\Lambda_{obs}-\Lambda_{ML})/[L]$			$(\Lambda_M-\Lambda_{obs})$			K_f			ΔG_f (k J/mol)		
	Cu	Co	Ni	Cu	Co	Ni	Cu	Co	Ni	Cu	Co	Ni	Cu	Co	Ni
0.002168	153.0003	77.87709	311.6939	0.03931	0.016292	0.05883	356.9997	412.1221	138.1061	9132.511	26295.58	2347.591	-23.8202	-26.4813	-20.2719
0.002053	148.7928	76.53127	307.0205	0.028399	0.01259	0.045439	363.2072	413.4687	142.9795	12789.27	32841.27	3146.609	-24.6999	-27.1692	-21.0371
0.001935	144.3875	74.42244	299.5928	0.018111	0.007727	0.028244	367.6125	415.5776	150.4072	20297.51	53780.36	5325.254	-25.9063	-28.4515	-22.4114
0.001803	140.1737	72.46812	293.5041	0.009276	0.003675	0.015335	371.8263	417.5319	156.4959	40066.58	113604.8	10205.02	-27.6839	-30.4048	-24.1103
0.001667	135.4038		286.2205	0.000623		0.002034	376.5962		163.7795	604486.3		80512.97	-34.7711		-29.5054

- The association free energies evaluated for CuCl2, CoCl2 and NiCl2 -1igand complexes indicating a spontaneous electrostatic attraction.

- The formation constants and Gibbs free energies of different complexes in absolute methanol at 313.15 K follow the order: K_f (2:1) › K_f (1:1) for (M:L), and ΔG_f(2:1) › ΔG_f(1:1) for (M:L) .

CONCLUSION

This work concentrated on the behavior of $CuCl_2$, $CoCl_2$ and $NiCl_2$ with the ligand conductometrically .The main target is to discuss the complexation between the metal and ligand for evaluating different concentrations from the metal ion in different solutions

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SYNTHESIS OF NEW SPIRO- HETEROCYCLES CONTAINING DIHYDROTETRAZINE MOIETY

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ABSTRACT

The reaction of nitrilimines with hydrazones of alkanones and cycloalkanones led to the formation of acyclic electrophilic addition products, which upon treatment with C/S/Zn cyclized to 1,6-dihydro-1,2,4,5-tetrazine derivatives. The structures of the synthesized compounds have been established by their elemental analyses and spectroscopical data.

KEYWORDS

Nitrilimines, Hydrazones, Cyclization, Synthesis, 1,6-dihydro-1,2,4,5-tetrazine

RESUMO

A reação de nitriliminas com hidrazonas de alcanonas e cicloalcanonas levou à formação de produtos acíclicos de adição eletrofílica. Depóis de tratamento com C/S/Zn eles levaram a derivados cíclicos de 1,6-dihidro-1,2,4,5-tetrazinas.As estruturas dos compostos sintetizados foram comprovadas com análise elementar e dados especroscópicos.

PALAVRAS CHAVE

Nitriliminas, Hidrazonas, Ciclização, Síntese de 1,6-dihidro-1,2.4,5-tetrazina

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GRAPHICAL ABSTRACT

Synthesis of New Spiro-Heterocycles Containing Dihydrotetrazine moiety

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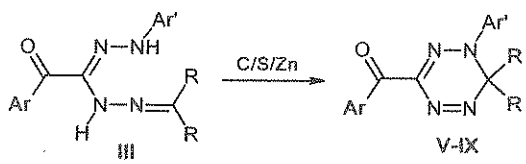


III V-IX

V, Ar = Ph; VI, Ar = PhNH; VII, Ar = 2-Furyl;
VIII, Ar = 2-Tienyl; IX, Ar = 2-Naphthyl

1. INTRODUCTION

Previous publications, showed that the simple hydrazones derived from aliphatic aldehydes and ketones react with nitrilimines at ambient temperature to give acyclic addition products, which undergo oxidative cyclization upon refluxing with active charcoal to yield the corresponding 1,6-dihydro-s-tetrazines [1] or amidrazones [2,3]. On the other hand, methyl hydrazones of alkanals and alkanones furnish 1,2,3,4-tetrahydro-s-tetrazines [3,4].

Recently, we found that nitrilimines react with 1-methyl, 1-phenyl, 1-acetyl, 1-formyl and 1-ethoxycarbonyl-1-methylhydrazines at room temperature afforded acyclic electrophilic addition products, which cyclized intramolecularly to the corresponding 1,2,3,4-tetrahydro-1,2,4,5-tetrazines by heating them with activated charcoal or lithium hydride in refluxing benzene or toluene [5.]

Quite recently, we described the synthesis of 1,2,3,4-tetrahydro-1,2,4,5-tetrazin-3-ones by the reaction of acetylhydrazone pyridinium chloride (Girard-reagent P) with different nitrilimines [6]. Several methods have been reported for the synthesis of tetrazine derivatives, and the most frequently used method for the preparation of 1,2,3,4-tetrahydro-1,2,4,5-tetrazines is the cyclization of alkylformazanes by heating or base treatment [7].

In the present study, the synthesis of a series of new substituted 1,2,4,5-tetrazines **5-9** were performed (Scheme 1) and their structures were characterized by ^{1}H NMR ^{13}C NMR, IR spectroscopy and elemental analysis.

2. RESULTS AND DISCUSSION

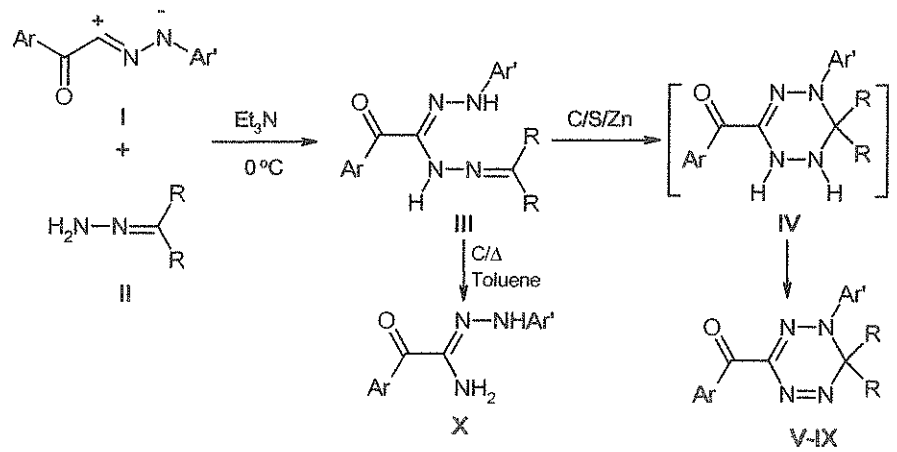
The formazans (acyclic adducts) **III** were synthesized via reaction of nitrilimines **I** with alkanones and cycloalkanone hydrazones **II** as shown in Scheme 1. Attempts to cyclize the acyclic adducts **III** (Ar = Me or OMe) by heating in tetrahydrofuran or ethanol were unsuccessful. However, treatment of solution of later adducts **III** (Ar = Me or OMe) with palladium-carbon brought about oxidative cyclization to the 1,6-dihydro-1,2,4,5-tetrazines [1].

On the other hand, cyclization of acyclic compounds **III** (Ar = Ph, PhNH, 2-furyl, 2-theinyl, 2-naphthyl) using active charcoal in refluxing toluene give complicated mixture of products as indicated by TLC, among which amidrazones **X** were separated, rather than the expected 1,6-dihydro-1,2,4,5-tetrazines [2,3] (Figure 1).

Treatment of solution of formazans **III** (Ar = Ph, PhNH, 2-furyl, 2-theinyl, 2-naphthyl) with new catalyst containing (C/S/Zn), developed in our laboratory by our colleague of physical chemistry, at room temperature in benzene or toluene give directly 1,6-dihydro-1,2,4,5-tetrazines **V-IX** (Figure 1) in excellent yields. (Table 1). It is suggested that the conversion of acyclic compounds **III** into s-tetrazines **V-IX** involves the non isolable intermediate formation of the tetrahydro-s-tetrazines **IV** (Figure 1).

2.1 Spectral data analysis

The assignment of structures of compounds V-IX is based on their analytical and spectroscopic data. Physical properties and microanalysis are presented in Table 1. These compounds gave satisfactory combustion analysis for the proposed structures which are confirmed on the basis of their spectroscopic data.



V, Ar = Ph; VI, Ar = PhNH; VII, Ar = 2-Furyl;
VIII, Ar = 2-Tienyl; IX, Ar = 2-Naphthyl

Ar' = 4-X-C_6H_4-

Entery	a	b	c	d	e	f	g	h	i	j	k	l
X	Cl	Cl	Cl	Cl	Cl	H	H	H	H	H	H	H
R R	Me Me					Me Me						

Figure 1. Synthetic pathway for the preparation of compounds V-IX.

In the IR spectra of compounds V-IX, showed the disappearance of NH signals and the C=O bond stretching of the carbonyl group at C-3 occurs at higher frequency (1665-1655 cm^{-1}) than it dose in the acyclic precursors III (1650-1635 cm^{-1}). This implies that conjugation of this exocyclic group with the hetero-ring π–system is decreased as a consequence of homoaromaticity and the slightly non-planar arrangement of the N-2, N-4 and C-3 plane with the substituents at C-3 [8-9] Compounds V-IX revealed strong absorption at about 1620-1600 cm^{-1} assigned to C=N bond stretching.

^1H and ^{13}C NMR spectra of obtained compounds V-IX provide strong evidence in support of the proposed structures. Their ^1H NMR spectra showed the disappearance of 2NH signals, in addition to aromatic protons signals, a characteristic signal due to amide NH proton for compounds VI resonating as singlet at 9.10-8.80 ppm. For compounds Va,VIa,VIf,IXa tow signals for the

methyl groups ($2CH_3$) protons appeared as singlet at 1.41-1.34 ppm and the signals of the cycloalkane protons in other compounds appeared in the range of 2.53-1.51 ppm.

The dihydrotetrazines **V-IX** exhibited a characteristic ^{13}C NMR signal at 68-87 ppm assigned to the C-6. This is similar to reported values of quaternary or spiro carbon flanked by tow nitrogens in six-membered heterocycles [2,3]. In the acyclic analogues **III**, this carbon resonates at 140-155 ppm [2,3]. This provides a strong evidence in support of cyclic structure of compounds **V-IX**. The ^1H and ^{13}C NMR spectral data of the synthesized compounds are presented in the experimental part.

3. EXPERIMENTAL SECTION

3.1. Reagents and Instrumentation

Triethylamine (TEA), tetrahydrofuran (THF), acetone, cyclohexanone, 4-methylcyclohexanone, 4-t-butylcyclohexanone, cyclopentanone, cycloheptanone, cyclooctanone and toluene were purchased from Avocado Chemical Company, England, and used as purchased. All melting points were determined on a Stuart Electrothermal Apparatus and are uncorrected.

The IR spectra were obtained by using Perkin-Elmer 737 infrared spectrophotometer in potassium bromide pellets. ^1H and ^{13}C NMR spectra were recorded on a Bruker spectrometer (400.13 MHz) at room temperature in $CDCl_3$ and DMSO-d_6, using tetramethylsilane (TMS) as an internal reference. All chemical shifts were reported as δ values in parts per million (ppm) downfield from internal TMS.

Electron impact (EI) mass spectra were measured on Shimadzu GCMS-QP1000 EX Mass spectrometers at 70 eV. Elemental analysis are performed at Cairo University, Egypt, and the results agreed with the calculated values within experimental errors. Nitrilimines **1** and hydrazones **2** used in this study, were prepared according to described procedures [1,10,11].

3.2. Synthesis of 1,6-dihydro-s-tetrazines V-IX

3.2.1 Reaction of nitrilimines I with hydrazones II

To a stirred mixture of the appropriate hydrazonoyl halide [nitrilimines I precursors] (0.01 mol) and hydrazones **II** (0.02 mol) in dry THF (100 mL), triethylamine (5 mL, 0.05 mol) in THF (20 mL) was dropwise added at -5 to 0 °C and the reaction mixture was controlled by TLC. The reaction temperature was allowed to rise slowly to room temperature and stirring was continued until the starting substrates were completely consumed (4-6 hours). The precipitated triethylammonium chloride salt was filtered off, the solvent was removed under reduced pressure. The residue was washed with water (3x50 mL), then triturated with ethanol (10 mL), the crude solid product was collected and recrystallized from aqueous ethanol to give the desired compounds **III**.

Table 1. Physical data and elemental analysis for compounds (V-IX).

Comp.	Molecular Formula (MW)	Yield (%)	mp (°C)	Analysis (%) Calculated / (Found)		
				C	H	N
Va	$C_{17}H_{15}ClN_4O$ (326.79)	82	173-5	62.48 (62.70)	4.63 (4.50)	17.14 (17.25)
Vb	$C_{19}H_{17}ClN_4O$ (352.83)	86	166-8	64.68 (64.45)	4.86 (4.75)	15.88 (16.05)
Vc	$C_{20}H_{19}ClN_4O$ (366.85)	89	184-6	65.48 (65.75)	5.22 (5.35)	15.27 (15.10)
Vd	$C_{21}H_{21}ClN_4O$ (380.88)	91	167-9	66.22 (61.95)	5.56 (5.70)	14.71 (14.60)
Ve	$C_{22}H_{23}ClN_4O$ (394.91)	87	175-7	66.91 (67.10)	5.87 (5.00)	14.19 (14.30)
VIa	$C_{17}H_{16}ClN_5O$ (341.80)	81	182-4	59.74 (60.00)	4.72 (7.65)	20.49 20.30)
VIc	$C_{20}H_{20}ClN_5O$ (381.87)	86	191-3	62.91 (63.15)	5.28 (5.35)	18.43 (18.60)
VIe	$C_{22}H_{24}ClN_5O$ (409.92)	84	187-9	64.46 (64.20)	5.90 (6.05)	17.08 (16.95)
VIf	$C_{17}H_{17}N_5O$ (307.36)	88	194-6	66.43 (66.65)	5.58 (5.40)	22.79 (22.90)
VIg	$C_{19}H_{19}N_5O$ (333.40)	92	201-3	68.45 (68.25)	5.74 (5.90)	21.01 (20.85)
VIh	$C_{20}H_{21}N_5O$ (347.42)	90	181-3	69.14 (68.90)	6.09 (5.95)	20.16 (20.30)
VIi	$C_{21}H_{32}N_5O$ (361.45)	87	196-8	69.78 (69.55)	6.41 (6.25)	19.38 (19.55)
VIj	$C_{24}H_{29}N_5O$ (403.53)	83	167-9	71.44 (71.15)	7.24 (7.35)	17.36 (17.20)
VIk	$C_{21}H_{23}N_5O$ (361.45)	94	177-9	69.78 (69.95)	6.41 (6.55)	19.38 (19.25)
VIl	$C_{22}H_{25}N_5O$ (375.48)	91	183-5	70.38 (70.10)	6.71 (6.55)	18.65 (18.80)
VIIb	$C_{17}H_{15}ClN_4O_2$ (342.74)	93	153-5	59.57 (59.80)	4.41 (4.30)	16.34 (16.20)
VIIc	$C_{18}H_{17}ClN_4O_2$ (356.81)	90	148-50	60.59 (60.35)	4.80 (4.95)	15.70 (15.85)
VIIIb	$C_{17}H_{15}ClN_4O_2S$ (358.85)	85	163-5	56.90 (57.15)	4.21 (4.40)	15.61 (15.50)
VIIId	$C_{19}H_{19}ClN_4O_2S$ (386.91)	89	146-8	58.98 (59.25)	4.95 (5.10)	14.48 (14.35)
IXa	$C_{21}H_{17}ClN_4O$ (376.85)	83	190-2	66.93 (67.20)	4.55 (4.40)	14.87 (15.00)
IXb	$C_{23}H_{19}ClN_4O$ (402.89)	91	176-8	68.57 (68.80)	4.75 (4.65)	13.91 (14.05)
IXc	$C_{24}H_{21}ClN_4O$ (416.91)	87	189-91	69.14 (68.90)	5.08 (4.95)	13.44 (13.60)
IXd	$C_{25}H_{23}ClN_4O$ (430.94)	84	168-70	69.68 (69.90)	5.38 (5.50)	13.00 (12.85)
IXe	$C_{26}H_{25}ClN_4O$ (444.97)	89	184-6	70.18 (69.95)	5.66 (5.80)	12.59 (12.45)

3.2.2 Cyclization of compounds (III):

Acyclic compounds III (0.005 mol) and C/S/Zn (0.1 w/w%) in benzene or toluene were stirred at room temperature for 1-2 hours and monitored by TLC. The reaction mixture was cooled, then filtered and the solvent was minimized and petroleum ether (bp. 40-60 °C) was slowly added to effect complete crystallization of the desired cyclic compounds V.
The following compounds were prepared using this method:

3-Benzoyl-1-(4-chlorophenyl)-6,6-dimethyl-1,6-dihydro-1,2,4,5-tetrazine (Va): ^{1}H NMR ($CDCl_3$) δ: 7.92-7.03 (m, 9H, Ar-CH), 1.39 (s, 3H, CH_3), 1.37 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 187.4 (C=O), 171.6 (COOH), 143.7 (C=N), 144.3-126.6 (Ar-C), 68.6 (quaternary carbon), 22.5 (CH_3). IR (KBr) *v*/cm^{-1}: 1660 (C=O), 159.2 (C=N).

8-Benzoyl-6-(4-chlorophenyl)-6,7,9,10-tetraazaspiro[4.5]dec-7,9-diene (Vb): ^{1}H NMR ($CDCl_3$) δ: 8.02-7.11 (m, 9H, Ar-CH), 1.90-1.68 (m, 8H, cyclopentane protons). ^{13}C NMR ($CDCl_3$) δ: 185.7 (C=O), 143.9 (C=N), 144.9-126.1 (Ar-C), 86.7 (spiro carbon), 32.1, 23.7 (cyclopentane carbons). IR (KBr) *v*/cm^{-1}: 1655 (C=O), 1594 (C=N).

3-Benzoyl-1-(4-chlorophenyl)-1,2,4,5-tetraazaspiro[5.5]undec-2,4-diene (Vc): ^{1}H NMR (DMSO-d_6) δ: 7.97-7.06 (m, 9H, Ar-CH), 1.86-1.66 (m, 10H, cyclohexane protons). ^{13}C NMR (DMSO-d_6) δ: 185.5 (C=O), 143.9 (C=N), 144.4-126.2 (Ar-C), 84.3 (spiro carbon), 32.1, 24.7, 23.4 (cyclohexane carbons). IR (KBr) *v*/cm^{-1}: 1655 (C=O), 1593 (C=N).

3-Benzoyl-1-(4-chlorophenyl)-1,2,4,5-tetraazaspiro[5.6]dodec-2,4-diene (Vd): ^{1}H NMR ($CDCl_3$) δ: 8.27-7.00 (m, 9H, Ar-CH), 2.53-1.56 (m, 12H, cycloheptane protons). ^{13}C NMR ($CDCl_3$) δ: 185.6 (C=O), 143.4 (C=N), 142.7-119.6 (Ar-C), 87.5 (spiro carbon), 39.5, 28.7, 22.3 (cycloheptane carbons). IR (KBr) *v*/cm^{-1}: 1660 (C=O), 1597 (C=N).

3-Benzoyl-1-(4-chlorophenyl)-1,2,4,5-tetraazaspiro[5.7]tridec-2,4-diene (Ve): ^{1}H NMR ($CDCl_3$) δ: 7.99-6.96 (m, 9H, Ar-CH), 2.46-1.36 (m, 14H, cyclooctane protons). ^{13}C NMR ($CDCl_3$) δ: 185.6 (C=O), 143.9 (C=N), 145.0-114.8 (Ar-C), 86.6 (spiro carbon), 34.4, 27.2, 25.2, 23.1 (cyclooctane carbons). IR (KBr) *v*/cm^{-1}: 1650 (C=O), 1594 (C=N).

1-(4-Chlorophenyl)-3-phenylaminocarbonyl-6,6-dimethyl-1,6-dihydro-1,2,4,5-tetrazine (VIa): ^{1}H NMR (DMSO-d_6) δ: 9.12 (s, 1H, NH), 7.61-7.18 (m, 10H, Ar-CH), 1.41 (s, 3H, CH_3), 1.38 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ: 159.2 (C=O amide), 136.7 (C=N), 142.4-126.6 (Ar-C), 68.7 (spiro carbon), 22.5 (CH_3). IR (KBr) *v*/cm^{-1}: 1650 (C=O), 1594 (C=N).

1-(4-Chlorophenyl)-3-phenylaminocarbonyl-1,2,4,5-tetraazaspiro[5.5]-undec-2,4-diene (VIc): ^{1}H NMR (DMSO-d_6) δ: 9.10 (s, 1H, NH), 7.63-7.20 (m, 10H, Ar-CH), 1.86-1.60 (m, 10H, cyclohexane protons). ^{13}C NMR (DMSO-d_6) δ: 159.3 (C=O amide), 136.9 (C=N), 141.9-125.8 (Ar-C), 84.8 (spiro carbon), 31.4, 25.7, 22.6 (cyclohexane carbons). IR (KBr) *v*/cm^{-1}: 1655 (C=O), 1598 (C=N).

1-(4-Chlorophenyl)-3-phenylaminocarbonyl-1,2,4,5-tetraazaspiro[5.7]-tridec-2,4-diene (VIe): ^{1}H NMR (DMSO-d_6) δ: 9.12 (s, 1H, NH), 7.60-7.20 (m, 14H, Ar-CH), 2.53-1.44 (m, 10H, cyclooctane protons). ^{13}C NMR (DMSO-d_6) δ: 159.4 (C=O amide), 136.8 (C=N), 141.7-126.0 (Ar-C), 86.5 (spiro carbon), 34.5,

30.7, 28.4, 23.2 (cyclooctane carbons). IR (KBr) ν/cm^{-1}: 1655 (C=O), 1596 (C=N).

1-Phenyl-3-phenylaminocarbonyl-6,6-dimethyl-1,6-dihydro-1,2,4,5-tetrazine (VIf): ^{1}H NMR (DMSO-d_6) δ: 9.00 (s, 1H, NH), 7.63-7.23 (m, 10H, Ar-CH), 1.37 (s, 3H, CH_3), 1.34 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ: 158.9 (C=O amide), 136.7 (C=N), 143.7-124.4 (Ar-C), 68.7 (spiro carbon), 22.7 (CH_3). IR (KBr) ν/cm^{-1}: 1650 (C=O), 1598 (C=N).

6-Phenyl-8-phenylaminocarbonyl-6,7,9,10-tetraazaspiro[4.5]dec-7,9-diene (VIg): ^{1}H NMR (DMSO-d_6) δ: 9.10 (s, 1H, NH), 7.60-7.19 (m, 10H, Ar-CH), 1.95-1.70 (m, 8H, cyclopentane protons). ^{13}C NMR (DMSO-d_6) δ: 158.8 (C=O), 136.4 (C=N), 142.3-126.2 (Ar-C), 86.6 (spiro carbon), 32.3, 23.4 (cyclopentane carbons). IR (KBr) ν/cm^{-1}: 1655 (C=O), 1596 (C=N).

1-Phenyl-3-phenylaminocarbonyl-1,2,4,5-tetraazaspiro[5.5]undec-2,4-diene (VIh): ^{1}H NMR (DMSO-d_6) δ: 9.00 (s, 1H, NH), 7.58-7.16 (m, 10H, Ar-CH), 1.85-1.63 (m, 10H, cyclohexane protons). ^{13}C NMR (DMSO-d_6) δ: 158.5 (C=O amide), 136.7 (C=N), 141.7-124.6 (Ar-C), 80.6 (spiro carbon), 32.0, 24.8, 23.1 (cyclohexane carbons). IR (KBr) ν/cm^{-1}: 1655 (C=O), 1595 (C=N).

9-Methyl-1-phenyl-3-phenylaminocarbonyl-1,2,4,5-tetraazaspiro[5.5]-undec-2,4-diene (VIi): ^{1}H NMR (DMSO-d_6) δ: 9.05 (s, 1H, NH), 7.62-7.17 (m, 10H, Ar-CH), 2.05-1.22 (m, 9H, cyclohexane protons), 0.94 (s, 3H, CH_3 at cyclohexane). ^{13}C NMR (DMSO-d_6) δ: 158.6 (C=O amide), 136.6 (C=N), 141.5-125.0 (Ar-C), 84.5 (spiro carbon), 33.8, 31.4, 28.4, 22.7 (methyl-cyclohexane carbons). IR (KBr) ν/cm^{-1}: 1655 (C=O), 1598 (C=N).

9-tert-Butyl-1-phenyl-3-phenylaminocarbonyl-1,2,4,5-tetraazaspiro-[5.5]undec-2,4-diene (VIj): ^{1}H NMR (DMSO-d_6) δ: 9.10 (s, 1H, NH), 7.66-7.21 (m, 10H, Ar-CH), 2.05-1.10 (m, 9H, cyclohexane protons), 0.88 (s, 9H, tert-butyl group) . ^{13}C NMR (DMSO-d_6) δ: 158.7 (C=O amide), 136.5 (C=N), 141.6-124.3 (Ar-C), 84.9 (spiro carbon), 47.1, 35.8, 32.4, 27.6, 24.1 (tert-butyl-cyclohexane carbons). IR (KBr) ν/cm^{-1}: 1650 (C=O), 1594 (C=N).

1-Phenyl-3-phenylaminocarbonyl-1,2,4,5-tetraazaspiro[5.6]dodec-2,4-diene (VIk): ^{1}H NMR (DMSO-d_6) δ: 8.95 (s, 1H, NH), 7.65-7.20 (m, 10H, Ar-CH), 2.45-1.62 (m, 12H, cycloheptane protons). ^{13}C NMR (DMSO-d_6) δ: 158.5 (C=O amide), 136.7 (C=N), 141.7-124.6 (Ar-C), 87.7 (spiro carbon), 39.6, 28.4, 22.3 (cycloheptane carbons). IR (KBr) ν/cm^{-1}: 1655 (C=O), 1596 (C=N).

1-Phenyl-3-phenylaminocarbonyl-1,2,4,5-tetraazaspiro[5.7]tridec-2,4-diene (VIl): ^{1}H NMR (DMSO-d_6) δ: 9.10 (s, 1H, NH), 7.60-7.20 (m, 10H, Ar-CH), 2.52-1.43 (m, 14H, cyclooctane protons). ^{13}C NMR (DMSO-d_6) δ: 158.4 (C=O amide), 136.5 (C=N), 139.7-126.6 (Ar-C), 86.9 (spiro carbon), 34.8, 31.1, 28.7, 23.2 (cyclooctane carbons). IR (KBr) ν/cm^{-1}: 1655 (C=O), 1593 (C=N).

6-(4-Chlorophenyl)-8-(2-furoyl)-6,7,9,10-tetraazaspiro[4.5]dec-7,9-diene (VIIb): ^{1}H NMR ($CDCl_3$) δ: 7.87-7.26 (m, 7H, Ar-CH), 1.95-1.70 (m, 8H, cyclopentane protons). ^{13}C NMR (DMSO-d_6) δ: 174.7 (C=O), 143.3 (C=N), 136.8-115.9 (Ar-C), 86.6 (spiro carbon), 34.5 32.2, 23.2 (cyclopentane carbons). IR (KBr) ν/cm^{-1}: 1665 (C=O), 1594 (C=N).

1-(4-Chlorophenyl)-3-(2-furoyl)-1,2,4,5-tetraazaspiro[5.5]undec-2,4-diene (VIIc): ^{1}H NMR ($CDCl_3$) δ: 8.26-7.21 (m, 7H, Ar-CH), 1.84-1.61 (m, 10H, cyclohexane protons). ^{13}C NMR (DMSO-d_6) δ: 174.6 (C=O), 143.1 (C=N), 136.7-

116.1 (Ar-C), 80.6 (spiro carbon), 32.6, 24.8, 23.3 (cyclohexane carbons). IR (KBr) ν/cm^{-1}: 1660 (C=O), 1595 (C=N).

6-(4-Chlorophenyl)-8-(2-thenoyl)-6,7,9,10-tetraazaspiro[4.5]dec-7,9-diene (VIIIb): ^{1}H NMR ($CDCl_3$) δ: 8.23-7.18 (m, 7H, Ar-CH), 1.92-1.67 (m, 8H, cyclopentane protons). ^{13}C NMR (DMSO-d_6) δ: 174.6 (C=O), 143.4 (C=N), 136.7-115.0 (Ar-C), 86.8 (spiro carbon), 32.4 23.7 (cyclopentane carbons). IR (KBr) ν/cm^{-1}: 1665 (C=O), 1598 (C=N).

1-(4-Chlorophenyl)-3-(2-thenoyl)-1,2,4,5-tetraazaspiro[5.6]dodec-2,4-diene (VIIId): ^{1}H NMR ($CDCl_3$) δ: 8.21-7.16 (m, 7H, Ar-CH), 2.42-1.60 (m, 12H, cycloheptane protons). ^{13}C NMR (DMSO-d_6) δ: 174.6 (C=O), 143.2 (C=N), 136.6-114.6 (Ar-C), 87.5 (spiro carbon), 39.5 28.2, 22.5 (cycloheptane carbons). IR (KBr) ν/cm^{-1}: 1665 (C=O), 1596 (C=N).

1-(4-Chlorophenyl)-6,6-dimethyl-3-(2-naphthoyl)-1,6-dihydro-1,2,4,5-tetrazine (IXa): ^{1}H NMR ($CDCl_3$) δ: 8.59-7.16 (m, 11H, Ar-CH), 1.41 (s, 3H, CH_3), 1.39 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ: 187.5 (C=O), 135.5 (C=N), 144.2-125.9 (Ar-C), 68.6 (spiro carbon), 22.6 (CH_3). IR (KBr) ν/cm^{-1}: 1645 (C=O), 1595 (C=N).

6-(4-Chlorophenyl)-8-(2-napthoyl)-6,7,9,10-tetraazaspiro[4.5]dec-7,9-diene (IXb): ^{1}H NMR ($CDCl_3$) δ: 8.57-7.12 (m, 11H, Ar-CH), 2.10-1.67 (m, 8H, cyclopentane protons). ^{13}C NMR (DMSO-d_6) δ: 187.4 (C=O), 135.4 (C=N), 144.0-115.3 (Ar-C), 80.7 (spiro carbon), 31.9, 23.5 (cyclopentane carbons). IR (KBr) ν/cm^{-1}: 1646 (C=O), 1598 (C=N).

1-(4-Chlorophenyl)-3-(2-naphthoyl)-1,2,4,5-tetraazaspiro[5.5]undec-2,4-diene (IXc): ^{1}H NMR ($CDCl_3$) δ: 8.56-7.13 (m, 11H, Ar-CH), 2.15-1.58 (m, 10H, cyclohexane protons). ^{13}C NMR ($CDCl_3$) δ: 187.5 (C=O), 135.5 (C=N), 143.9-126.0 (Ar-C), 70.9 (spiro carbon), 30.8, 25.8, 22.6 (cyclohexane carbons). IR (KBr) ν/cm^{-1}: 1648 (C=O), 1597 (C=N).

1-(4-Chlorophenyl)-3-(2-naphthoyl)-1,2,4,5-tetraazaspiro[5.6]dodec-2,4-diene (IXd): ^{1}H NMR ($CDCl_3$) δ: 8.58-7.24 (m, 11H, Ar-CH), 2.35-1.65 (m, 12H, cycloheptane protons). ^{13}C NMR ($CDCl_3$) δ: 187.1 (C=O), 135.6 (C=N), 144.5-119.6 (Ar-C), 85.3 (spiro carbon), 39.4, 31.2, 28.3, 22.6 (cycloheptane carbons). IR (KBr) ν/cm^{-1}: 1645 (C=O), 1593 (C=N).

1-(4-Chlorophenyl)-3-(2-naphthoyl)-1,2,4,5-tetraazaspiro[5.7]tridec-2,4-diene (IXe): ^{1}H NMR ($CDCl_3$) δ: 8.56-6.98 (m, 11H, Ar-CH), 2.48-1.37 (m, 14H, cyclooctane protons). ^{13}C NMR ($CDCl_3$) δ: 187.2 (C=O), 135.7 (C=N), 142.1-114.7 (Ar-C), 84.9 (spiro carbon), 34.6, 30.8, 28.7, 23.4 (cyclooctane carbons). IR (KBr) ν/cm^{-1}: 1645 (C=O), 1594 (C=N).

4. CONCLUSION

In conclusion, the results demonstrate that the nitrilimines react with hydrazone of aliphatic alkanones and cycloalkanone to give an acyclic addition product, which upon treatment with new catalyst (C/S/Zn) yielded the spiro heterocyclic compounds containing tetrazine moiety.

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SYNTHESIS AND ANTIMICROBIAL PROFILE OF SOME NEWER HETEROCYCLES BEARING THIAZOLE MOIETY

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ABSTRACT

Various substituted acetophenones on treatment with iodine and thiourea yielded 2-amino-4-(substituted-phenyl)-thiazole, which on further treatment with acetic anhydride generated *N*-(4-(substitutedphenyl)thiazol-2-yl)acetamide (1-5). All the synthesized compounds were characterized by their respective FTIR, ^{1}H NMR and mass data. Synthesized compounds (1, 2, 3, 4, 5) when subjected to investigation for their antimicrobial activities i.e. antibacterial and antifungal studies against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Asperigillus flavus and Asperigillus fumigatus* by disk diffusion method, revealed that compound 2 deemed to be most potent with largest zone of inhibition.

KEYWORDS: Thiazole, Acetophenones, Antimicrobial, Substituted Aldehydes.

RESUMO

Tratamento de acetofenonas substituídas com iodo e tiouréia levou a formação de vários 2-amino tiazóis -4- (fenilsubstituidos) . O tratamento destes com anihidrido acético gerou N-(4-fenilsubstituido)tiazol-2-il) acetamidas (1-5). Todos os compostos sintetizados foram caracterizados com técnicas de infravermelho com transformadas de Fourier, RMN de ^{1}H e espectrometria de massa. .As propriedades frmacêuticas dos compostos 1,2,3,4 e 5 foram avaliadas com *Staphylococcus aureus, Eschericia coli, Pseudomonas aeruginosa, Cândida albicans, Aspergillus flavus e Aspergillus fumigatus.* O composto 2 foi o mais potente.

PALAVRAS CHAVE: Tiazol, Acetofenonas, Aldeídos Substituídos, Atividade Antimicrobiana.

INTRODUCTION

Thiazole derivatives have attracted a great deal of interest owing to their anticancer activity[1-3], antibacterial activity[4], antifungal activity[4], anti-inflammatory activity[4], antitubercular activity[5], cardiotonic activity[6], antidegenerative activity on cartilage[7] etc. Thiazoles are known to be allosteric enhancer of A_1 adenosine receptors[8] whereas other analogs are known to be inhibitors of protein phosphatases[9]. Heterocycle-bearing substrates are particularly desirable structures for screening and are prevalent in drugs that have reached the market place.

The development of simple and general synthetic routes for widely used organic compounds from readily available reagents is one of the major challenges in organic chemistry. Therefore to meet the facile results of these tough challenges thiazole nucleus was being considered. Among the wide variety of heterocycles that have been explored for developing pharmaceutically molecules, thiazole derivatives have played a vital role in the medicinal chemistry. There are large numbers of synthetic compounds with thiazole nucleus used for anticancer activities when properly substituted at 2-position. In view of these observations and in continuation to develop better and potent anticancer agents, some newer thiazole derivatives were synthesized.

MATERIALS AND METHODS

Melting points were taken in open capillaries and are uncorrected. IR spectrum of compounds in KBr pellets were recorded on a FTIR-8400S spectrophotometer (SHIMADZU). ^{1}HNMR spectra of the compounds were recorded on Bruker DRX 300 NMR spectrophotometer in DMSO-d_6 using TMS as internal standard. Mass spectra of the compounds were recorded on MSN-9629 mass spectrometer. Elemental analysis was carried out on Elemental Vario EL III Carlo Erba 1108. The purity of compounds was monitored by thin layer chromatography. Thin layer chromatographic analysis of the compounds were performed on silica gel G coated glass plates using Chloroform: Methanol: Pet.Ether (9:1:0.5) as mobile phase. The spots were visualized by exposure to iodine vapours.

General method for the synthesis of 2-amino-4-(substituted-phenyl)-thiazole

Various substituted acetophenones (0.01mol) were refluxed with iodine (0.01mol) and thiourea (0.02mol) for 9 hrs to get 2-amino-4-(substituted-phenyl)thiazole. The solid obtained was washed with diethyl ether, after which it was washed with sodium thiosulfate. Finally, it was washed with water and the residue was filtered, dried and recrystallized from distilled water.

General method for the synthesis of (1-5)

Then, 2-amino-4-(substituted-phenyl)thiazole (0.01mol) was refluxed with acetic anhydride (0.01mol) for 2hrs. This led to the formation of N-(4-(substituted-phenyl)thiazol-2-yl)acetamide (1-5). The final products were purified by recrystallization from ethanol. Physical data of compounds synthesized are summarized in Table-1.

Table-1. Physical data of compounds (1-5)

Compound	R	Molecular Formula	Mol. Wt.	Yield (%)	m.p. (°C)
1	H	$C_{11}H_{10}N_2OS$	218.27	61	98-99
2	*p*-chloro	$C_{11}H_9ClN_2OS$	252.72	69	209-210
3	*p*-bromo	$C_{11}H_9BrN_2OS$	297.17	65	202-203
4	*p*-hydroxy	$C_{11}H_{10}N_2O_2S$	234.27	65	141-142
5	*o*-hydroxy	$C_{11}H_{10}N_2O_2S$	234.27	70	115-116

***N*-(4-phenylthiazol-2-yl)acetamide (1):** UV λ_{max} (Methanol): 232 nm. FTIR (KBr): 3392.55 (N-H stretching), 2977.89 (aromatic C-H stretching), 2931.6 (C-H stretching of methyl), 1622.02 (C=O stretching), 1569.95 (C=N stretching), 1498.59 (aromatic C-C stretching), 690.47 cm^{-1} (C-S stretching of thiazole). ^{1}HNMR (DMSO-d_6) δ : 2.142 (s, 3H, CH_3), 7.117 (s, 1H, =C-H of thiazole), 7.273-7.854 (m, 5H, Ar-H), 8.854 ppm (s, 1H, NH, D_2O exchangeable). ESI-MS: m/z (%) 219 (8) $[M+1]^+$, 218 (43) $[M]^+$, 203 (40), 175 (100), 134 (43), 133 (23). Elemental Analysis: Calcd for $C_{11}H_{10}N_2OS$: C, 60.53; H, 4.62; N, 12.83; S, 14.69. Found: C, 60.50; H, 4.63; N, 12.81; S, 14.68 %.

***N*-(4-(4-chlorophenyl)thiazol-2-yl)acetamide (2):** UV λ_{max} (Methanol): 224 nm. FTIR (KBr): 3394.48 (N-H stretching), 2981.74 (aromatic C-H stretching), 2947.03 (C-H stretching of methyl), 1623.95 (C=O stretching), 1564.16 (C=N stretching), 1492.8 (aromatic C-C stretching), 746.4 (C-Cl stretching), 651.03 cm^{-1} (C-S stretching of thiazole). ^{1}HNMR (DMSO-d_6) δ : 2.466 (s, 3H, CH_3), 6.545 (s, 1H, =C-H of thiazole), 7.116-7.625 (m, 4H, Ar-H), 9.154 ppm (s, 1H, NH, D_2O exchangeable). ESI-MS: m/z (%) 254 (17) $[M+2]^+$, 253 (6) $[M+1]^+$, 252 (46) $[M]^+$, 237 (32), 209 (100), 168 (42), 167 (22). Elemental Analysis: Calcd for $C_{11}H_9ClN_2OS$: C, 52.28; H, 3.59; Cl, 14.03; N, 11.08; S, 12.69. Found: C, 52.27; H, 3.57; Cl, 14.02; N, 11.06; S, 12.71 %.

***N*-(4-(4-bromophenyl)thiazol-2-yl)acetamide (3):** UV λ_{max} (Methanol): 225 nm. FTIR (KBr): 3417.63 (N-H stretching), 3029.33 (aromatic C-H stretching), 2993.32 (C-H stretching of methyl), 1672.17 (C=O stretching), 1598.88 (C=N stretching), 1488.94 (aromatic C-C stretching), 693.26 (C-S stretching of thiazole), 570.89 cm^{-1} (C-Br stretching). ^{1}HNMR (DMSO-d_6) δ : 2.763 (s, 3H, CH_3), 6.967 (s, 1H, =C-H of thiazole), 7.317-7.825 (m, 4H, Ar-H), 8.778 ppm (s, 1H, NH, D_2O exchangeable). ESI-MS: m/z (%) 299 (43) $[M+2]^+$, 298 (8) $[M+1]^+$, 297 (42) $[M]^+$, 282 (40), 254 (100), 213 (32), 212 (27). Elemental Analysis: Calcd for $C_{11}H_9BrN_2OS$: C, 44.46; H, 3.05; Br, 26.89; N, 9.43; S, 10.79. Found: C, 44.45; H, 3.01; Br, 26.87; N, 9.46; S, 10.80 %.

***N*-(4-(4-hydroxyphenyl)thiazol-2-yl)acetamide (4):** UV λ_{max} (Methanol): 242 nm. FTIR (KBr): 3558.42 (O-H stretching), 3406.05 (N-H stretching), 3048.29 (aromatic C-H stretching), 2923.88 (C-H stretching of methyl), 1631.67 (C=O stretching), 1554.52 (C=N

stretching), 1526.93 (aromatic C-C stretching), 675.04 cm^{-1} (C-S stretching of thiazole). ^{1}HNMR (DMSO-d_6) δ: 2.228 (s, 3H, CH_3), 4.955 (s, 1H, OH, D_2O exchangeable), 6.369 (s, 1H, =C-H of thiazole), 7.296-7.658 (m, 4H, Ar-H), 8.564 ppm (s, 1H, NH, D_2O exchangeable). ESI-MS: m/z (%) 235 (6) $[M+1]^+$, 234 (28) $[M]^+$, 219 (22), 191 (100), 150 (20), 149 (14). Elemental Analysis: Calcd for $C_{11}H_{10}N_2O_2S$: C, 56.39; H, 4.30; N, 11.96; S, 13.69. Found: C, 56.40; H, 4.33; N, 11.98; S, 13.65 %.

***N*-(4-(2-hydroxyphenyl)thiazol-2-yl)acetamide (5):** UV λ_{max} (Methanol): 267 nm. FTIR (KBr): 3555.6 (O-H stretching), 3408.42 (N-H stretching), 3046.05 (aromatic C-H stretching), 2926.05 (C-H stretching of methyl), 1633.88 (C=O stretching), 1554.07 (C=N stretching), 1523.96 (aromatic C-C stretching), 1291.67 (C-O stretching), 673.68 cm^{-1} (C-S stretching of thiazole). ^{1}HNMR (DMSO-d_6) δ: 2.156 (s, 3H, CH_3), 4.702 (s, 1H, OH, D_2O exchangeable), 6.911 (s, 1H, =C-H of thiazole), 7.316-7.625 (m, 4H, Ar-H), 8.778 ppm (s, 1H, NH, D_2O exchangeable). ESI-MS: m/z (%) 235 (6) $[M+1]^+$, 234 (28) $[M]^+$, 219 (22), 191 (100), 150 (20), 149 (14). Calcd for $C_{11}H_{10}N_2O_2S$: C, 56.39; H, 4.30; N, 11.96; S, 13.69. Found: C, 56.37; H, 4.33; N, 11.98; S, 13.67 %.

Antimicrobial activity

The synthesized compounds 1-5 were screened for antibacterial (*S. aureus, E. coli, P. aeruginosa*) and antifungal (*C. albicans, A. flavus, A. fumigatus*) activities by disk diffusion method at a concentration of 2 mg/mL using DMF as a solvent. The results were recorded in duplicate using Ciprofloxacin and Fluconazole as standards and are given in Table 2 & 3.

Table-2: Antibacterial Activity of compounds (1-5)

Compounds	Zone of Inhibition (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
1.	15.5 ± 0.00	17 ± 0.33	16 ± 0.00
2.	21.5 ± 0.33	20.5 ± 0.00	19.3 ± 0.00
3.	19.7 ± 0.67	20.3 ± 0.33	20.3 ± 0.33
4.	16.4 ± 0.00	15 ± 0.00	15.5 ± 0.00
5.	17.3 ± 0.00	17 ± 0.33	17 ± 0.67
Ciprofloxacin	27 ± 0.00	28 ± 0.00	27 ± 0.00
DMF	-	-	-

Table 3. Antifungal Activity of Compounds (1-5)

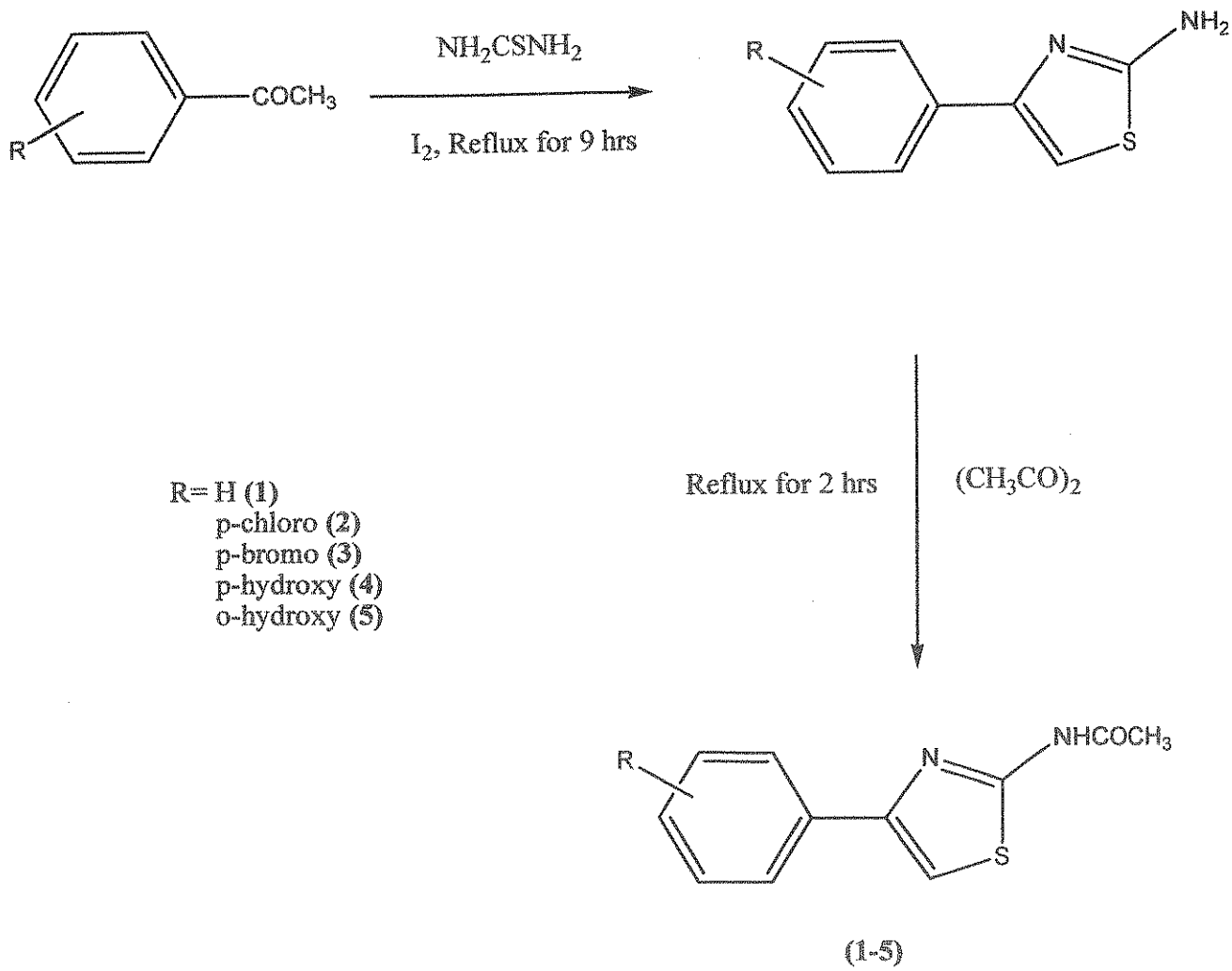
Compounds	Zone of Inhibition (mm)		
	C. albicans	*A. flavus*	*A. fumigatus*
1.	5.4 ± 0.00	5.0 ± 0.00	6.5 ± 0.00
2.	11.3 ± 0.33	12.5 ± 0.00	11 ± 0.00
3.	10.7 ± 0.67	9.3 ± 0.33	8.3 ± 0.33
4.	8.2 ± 0.00	7.4 ± 0.00	8.0 ± 0.00
5.	8.3 ± 0.00	7.8 ± 0.00	8.7 ± 0.67
Fluconazole	17 ± 0.00	16 ± 0.00	17 ± 0.00
DMF	-	-	-

RESULTS AND DISCUSSION

Various substituted acetophenones reacted with iodine and thiourea to get 2-Amino-4-(substituted-phenyl)-thiazole[10]. Nextly, the 2-amino group of 2-Amino-4-(substituted-phenyl)-thiazole was acetylated with acetic anhydride, which led to the formation of *N*-(4-(substitutedphenyl)thiazol-2-yl)acetamide (**1-5**) in moderate to good yields (Scheme-1). The FTIR spectra of compounds **1-5** exhibited bands in the region of 3344.12-3417.23 cm^{-1} due to N-H stretching and in the region 1622.02-1672.46 cm^{-1} due to C=O stretching of amide. In ^1H NMR spectra of compounds **1-5**, one proton singlet appeared between δ 8.85-9.15 ppm was assigned to N-H proton which disappeared on D_2O exchange.

The structures of the synthesized compounds were assigned on the basis of elemental analysis, ^1H NMR, FTIR and mass spectral data and physical data. The synthesized compounds 1-5 were screened for antibacterial (*S. aureus, E. coli, P. aeruginosa*) and antifungal (*C. albicans, A. flavus, A. fumigatus*) activities by disk diffusion method at a concentration of 2 mg/mL using DMF as a solvent. This revealed that compound **2** deemed to be most potent with the largest zone of inhibition for both i.e. antibacterial activity and antifungal Activity.

SCHEME 1:



R= H (**1**)
p-chloro (**2**)
p-bromo (**3**)
p-hydroxy (**4**)
o-hydroxy (**5**)

(**1-5**)

Where R = H, *p*-chloro, *p*-bromo, *p*-hydroxy and *o*-hydroxy- group

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SYNTHESIS, CHARACTERIZATION AND COMPARATIVE SCREENING OF SOME NEWER 2-PHENYL INDOLE AND 5-CHLORO-2-PHENYL INDOLE DERIVATIVES

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ABSTRACT
Biologically active phenyl indole and chloro phenyl indole derivatives were efficiently synthesized. The reaction of 2-phenyl-1H-indole **A** and 5-chloro-2-phenyl-1H-indole **B**, with chloroacetylchloride yielded 2-chloro-1-(2-phenyl-1H-indol-1yl)ethanone **1** and 2-chloro-1-(5-chloro-2-phenyl-1H-indol-1-yl)ethanone **4** respectively. Compound **1** and **4** on Friedal Crafts cyclization in presence of aluminium chloride and nitrobenzene yielded indolo[2,1-α]isoquinolin-6(5H)-one **2** and 10-chloroindolo [2,1-α]isoquinolin-6(5H)-one **5** respectively, which upon hydrolysis afforded 2-(2-(1H-indol-2-yl)phenyl)acetic acid **3** and 2-(2-(5-chloro-1H-indol-2-yl) phenyl) acetic acid **6** respectively. The newly designed compounds were characterized on the basis of spectral studies and screened for anti-inflammatory and anti-microbial activities.
KEYWORDS: 2-phenyl-indole, 5-chloro-2-phenyl-indole, Friedal Crafts cyclization.

RESUMO
Derivados de fenil indol e clorofenil indol, biologicamente ativos, foram sintetizados de maneira eficiente. A reação de 2-fenil- 1H-indol e 5-cloro-2-fenil-1H-indole com cloreto de cloroacetila seguida por ciclização Friedel Crafts levou aos compostos **2** e **5**, respectivamnete, os quais depois de hidrólise formaram 2-(2-(1H-indol-2-il)ácido fenilacético,**3**, e 2-(2-(5-cloro-1H-indol-2-il) ácido fenilacético, **6**. Os compostos foram caracterizados e a atividade antiinflamatória e antimicrobial foram avaliadas.
PALAVRAS CHAVE: 2-Fenil indol, 5-Cloro-2-fenil indol, Ciclização Friedel Crafts

INTRODUCTION

The statistical data provided that the global pharmaceutical market *grew to 712 billion* US dollars *in 2007* at a rate of *10.7% and is* expected to grow to 929 billion US dollars by 2012, which consists of 25.5 billion dollars of NSAIDS market. The global anti-infective market is currently valued at 66.5 *billion* US dollars with antibacterial agents accounting for over 50% of sales. Indole and phenyl acetic acid derivatives are known to have potent anti –inflammatory (1), anti-microbial (2) and analgesic (3) activities. As per prospects of NSAIDS in global pharmaceutical market and literary evidences for activities associated with indoles, an attempt was made to generate novel potent anti-inflammatory and anti-microbial drugs by converting a 2-phenyl indole moiety **A** and 5-chloro-2- phenyl indole moiety **B** into some novel 2-(2-(1H-indol-2-yl)phenyl)acetic acid **3** and 2-(2-(5-chloro-1H-indol-2-yl) phenyl) acetic acid **6.** During this pathway of synthesis of 2-chloro-1-(2-phenyl-1H-indol-1yl)ethanone **1**, indolo[2,1-α]isoquinolin-6(5H)-one **2**, 2-chloro-1-(5-chloro-2-phenyl-1H-indol-1yl)ethanone **4** and10-chloroindolo [2,1-α]isoquinolin-6(5H)-one **5** were obtained as key intermediates. All the newly designed compounds were further characterized and evaluated for anti-inflammatory and anti-microbial activities.

EXPERIMENTAL

Melting points of newly designed compounds were determined in open capillary tubes. IR spectra were recorded (in KBr) on Perkin Elmer and 1HNMR spectra on Bruker, SF 300 instruments. Purity of designed compounds was checked by TLC on aluminium sheets with silica gel 60 F254 (0.2 mm).

2-chloro-1-(2-phenyl-1H-indol-1yl)ethanone (1)

To a solution of 2-phenyl-1H-indole **A** (0.01 mol) in methyl ethyl ketone, a solution of chloro acetyl chloride (in methyl ethyl ketone) was added dropwise on a magnetic stirrer. During the reaction to maintain the pH 8-9 a solution of sodium carbonate (in distilled water) was also added dropwise. The stirring was continued for further 75 min. From the resultant mixture the organic layer was separated and subjected for distillation under reduced pressure. The obtained crude product was recrystallized from methanol to yield compound **1**.

IR (KBr, cm^{-1}): 2916 (C-H of CH_2), 3020 (C-H of aromatic ring), 1662 (C=O of amide)

^{1}HNMR (CDCl3, ppm): 4.88 (2H; s; CH_2), 6.53 (1H; s; H_3), 7.05-7.29 (3H; m; H_5, H_6 & H_4'), 7.31-7.46 (5H; m; H_7, H_2', H_3', H_5', H_6'), 7.6 (1H; m; H_4)

MS (m/z): 269 (M^+), 233, 76, 51

indolo[2,1-α]isoquinolin-6(5H)-one (2)

To a solution of 2-chloro-1-(2-phenyl-1H-indol-1yl)ethanone **1** in nitrobenzene, 1g of powdered aluminium chloride was added in small portions with simultaneous stirring for 15 min. The reaction mixture was further stirred continuously for 1 hr. The resultant mixture was transferred onto crushed ice to form a semisolid mass, which was subjected to distillation to remove nitrobenzene to get a solid product. The obtained crude product was recrystallized from methanol to yield compound **2**

IR (KBr, cm-1): 2922 (C-H of CH_2), 3045 (C-H of aromatic ring), 1668 (C=O of amide)
^{1}HNMR (CDCl3, ppm): 3.66 (2H, s, CH_2), 6.61 (1H; s; H_3), 6.8-7.24 (5H; m; H_5, H_6 H_3', H_4', & H_5'), 7.29-7.67 (3H; m; H_4, H_5, H_2')
MS (m/z): 233 (M^+), 76, 51

2-(2-(1H-indol-2-yl)phenyl)acetic acid (3)
A mixture of indolo[2,1-α]isoquinolin-6(5H)-one 2 in ethanol and sodium hydroxide solution was refluxed for 6 hrs. The resultant reaction mixture was filtered and to the filtrate HCl was added drop wise to yield a solid mass. The crude product so obtained was filtered and recrystallized from methanol to yield 2-(2-(1H-indol-2-yl)phenyl)acetic acid 3.
IR (KBr, cm-1): 3447 (O-H of COOH), 3021 (C-H of aromatic ring), 2930 (C-H of methylene), 1721 (C=O of COOH),
^{1}HNMR (CDCl3, ppm): 8.52 (1H, s, N-H), 3.42 (2H, s, CH_2), 6.51 (1H, s, H_3), 6.82-7.19 (5H; m; H_5, H_6, H_3', H_4', H_5'), 7.29-7.64 (3H, m; H_2', H_7, H_4), 11.2 (1H, s, O-H)
MS (m/z): 251 (M^+), 234, 233, 224, 206, 91, 76, 51, 45

2-chloro-1-(5-chloro-2-phenyl-1H-indol-1yl)ethanone (4)
To a solution of 5-chloro-2-phenyl-1H-indole A (0.01 mol) in methyl ethyl ketone, a solution of chloro acetyl chloride (in methyl ethyl ketone) was added dropwise on a magnetic stirrer. During the reaction to maintain the pH 8-9 a solution of sodium carbonate (in distilled water) was also added drop wise. The stirring was continued for further 75 min. From the resultant mixture the organic layer was separated and subjected for distillation under reduced pressure. The obtained crude product was recrystallized from methanol to yield compound 4.
IR (KBr, cm-1): 2919 (C-H of CH_2), 3028 (C-H of aromatic ring), 1664 (C=O of amide)
^{1}HNMR (CDCl3, ppm): 4.92 (2H; s; CH_2), 6.59 (1H; s; H_3), 7.14-7.34 (3H; m; H_5, H_6 & H_4'), 7.39-7.49 (5H; m; H_7, H_2', H_3', H_5', H_6'), 7.54 (1H; d; j=2.7, H_4)
MS (m/z): 303 (M^+), 267, 76, 51

10-chloroindolo [2,1-α]isoquinolin-6(5H)-one (5)
To a solution of 2-chloro-1-(5-chloro-2-phenyl-1H-indol-1yl)ethanone 4 in nitrobenzene, 1g of powdered aluminium chloride was added in small portions with simultaneous stirring for 15 min. The reaction mixture was further stirred continuously for 1 hr. The resultant mixture was transferred onto crushed ice to form a semisolid mass, which was subjected to distillation to remove nitrobenzene to get a solid product. The obtained crude product was recrystallized from methanol to yield compound 5.
IR (KBr, cm-1): 2930 (C-H of CH_2), 3049 (C-H of aromatic ring), 1674 (C=O of amide)
^{1}HNMR (CDCl3, ppm): 3.72 (2H, s, CH_2), 6.68 (1H; s; H_3), 6.94-7.28 (5H; m; H_5, H_6 H_3', H_4', & H_5'), 7.36-7.48 (2H; m; H_5, H_2'), 7.56 (1H; d; j=2.6, H_4)
MS (m/z): 267 (M^+), 231, 91, 76, 51

2-(2-(5-chloro-1H-indol-2-yl) phenyl) acetic acid (6)
A mixture of 10-chloroindolo [2,1-α]isoquinolin-6(5H)-one 5 in ethanol and sodium hydroxide solution was refluxed for 6 hrs. The resultant reaction mixture was filtered

and to the filtrate HCl was added drop wise to yield a solid mass. The crude product so obtained was filtered and recrystallized from methanol to yield 2-(2-(5-chloro-1H-indol-2-yl) phenyl) acetic acid **6**.

IR (KBr, cm-1): 3458 (O-H of COOH), 3028 (C-H of aromatic ring), 2942 (C-H of methylene), 1716 (C=O of COOH)

^{1}HNMR (CDCl3, ppm): 8.65 (1H, s, N-H), 3.52 (2H, s, CH_2), 6.47 (1H, s, H_3), 6.93-7.14 (4H; m; H_6, H_3', H_4', H_5') 7.32-7.38 (2H; m; H_7, H_2') 7.62 (1H; d; J = 2.8, H_4), 11.35 (1H; s, O-H)

MS (m/z): 285 (M^+), 268, 267, 258, 249, 240, 91, 76, 51, 45

Biological activity

The designed compounds **1, 2, 3, 4, 5, 6** were screened for anti-inflammatory activity by carageenan induced paw oedema method using distilled water as solvent. The results were recorded using indomethacin as standard drug and are given in table-II. The designed compounds **1-6** were also were screened for antibacterial and antifungal activity using disk diffusion method. The results were recorded using amoxicillin and egriseofulvin as standard drugs respectively and are given in Table-III and Table-IV.

RESULTS AND DISCUSSION

2-chloro-1-(2-phenyl-1H-indol-1yl)ethanone **1** and 2-chloro-1-(5-chloro-2-phenyl-1H-indol-1-yl)ethanone **4**, prepared from 2-phenyl-1H-indole **A** and 5-chloro-2-phenyl-1H-indole **B** respectively. The obtained compounds **1** and **4** when cyclized with aluminium chloride yielded indolo[2,1-α]isoquinolin-6(5H)-one **2** and 10-chloroindolo [2,1-α]isoquinolin-6(5H)-one **5** respectively, which on hydrolysis lead to potent anti-inflammatory 2-(2-(1H-indol-2-yl)phenyl)acetic acid **3** and 2-(2-(1H-indol-2-yl)phenyl)acetic acid **6** respectively. The synthetic procedure for conversion of compound **A to 3** and **B to 6** is suggested in Scheme 1 and 2. Physical data of **1-6** are given in Table I. The assigned structure, molecular formulae and the anomeric configuration of the newly designed compounds **1-3** and **4- 6** were further confirmed and supported by mass, 1H-NMR and IR spectral data, based on occurrence of molecular ion peak of the assigned structures, downfield shifting of protons and different stretching bands of the compounds. The resultant compounds 1, 2, 3, 4, 5 and 6 after characterizations were further screened for anti-inflammatory and anti-microbial activity (data given in Table-II, III and IV).

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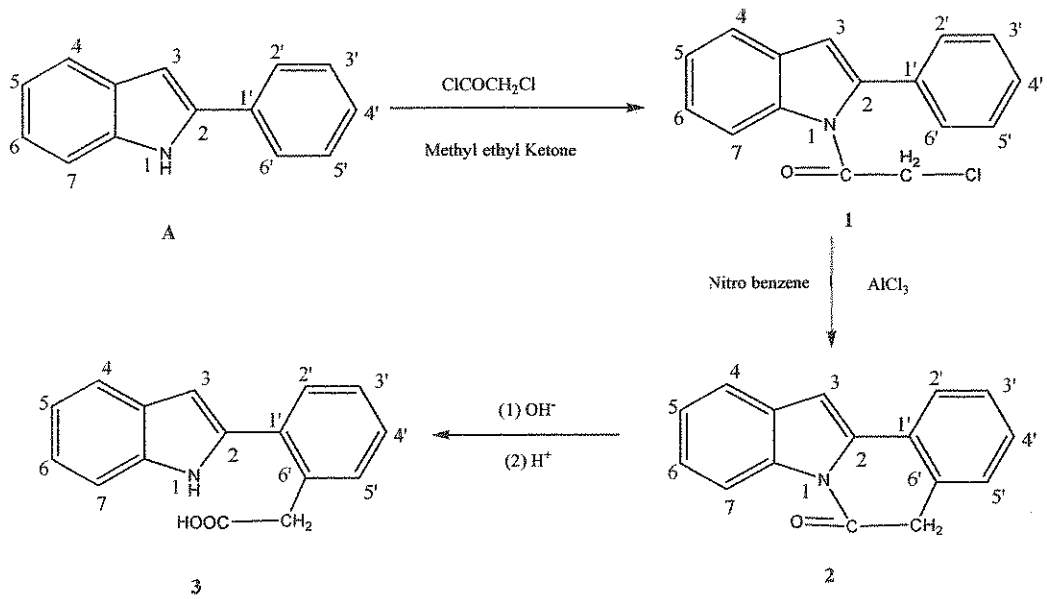
Scheme1



Scheme 2

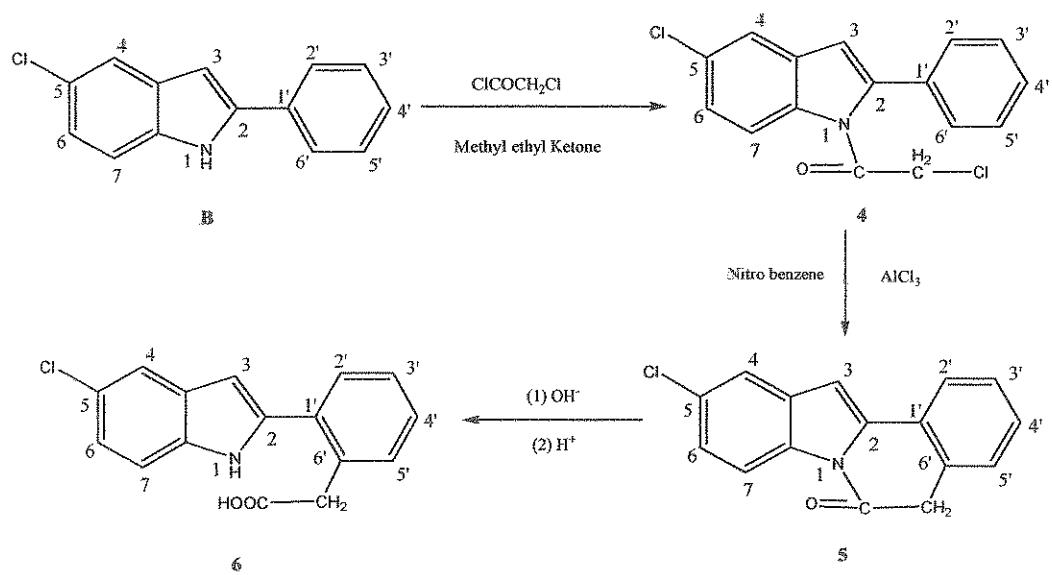


Table I. Physical data of compounds

Compound No.	Physical characteristics	Yield (%)	Molecular formula	Mol. Wt.	M.P. (°C)	R_f Value
1	White crystals	82	$C_{16}H_{12}ClNO$	269.73	205-206	0.56
2	White crystals	76	$C_{16}H_{11}NO$	233.26	212-213	0.42
3	White crystals	73	$C_{16}H_{13}NO_2$	251.28	228-229	0.38
4	White crystals	74	$C_{16}H_{11}Cl_2NO$	304.17	217-218	0.52
5	White crystals	68	$C_{16}H_{10}ClNO$	267.71	223-224	0.46
6	White crystals	64	$C_{16}H_{12}ClNO_2$	285.72	231-232	0.34

TABLE II- Anti-inflammatory activity of 2-phenyl indole and 5-chloro-2-phenyl indole derivatives on carrageenan-induced paw oedema in rats.

Compd 20mg/po	Paw volume in ml, mean ±SD(% inhibition of paw edema)			
	After 1hr	After 2hr	After 3hr	After 4hr
Control	0.880±0.0179	0.886±0.0163	0.897±0.0151	0.885±0.0242
Indomethacin	0.368±0.0197 (58.18%)*	0.326±0.0163 (63.2%)*	0.290±0.0219 (67.67)*	0.265±0.0350 (70.05%)*
1	0.847±0.0242 (3.75%)	0.833±0.0273 (5.98%)	0.803±0.029 (10.47%)	0.790±0.0452 (10.73%)
2	0.840±0.0219 (4.45%)	0.817±0.0151 (7.78%)	0.787±0.0350 (12.26%)	0.757±0.0234 (14.46%)
3	0.583±0.0408 (33.75%)*	0.557±0.0197 (37.13%)*	0.527±0.0350 (41.24%)*	0.503±0.067 (43.16%)*
4	0.817±0.0388 (7.15%)	0.793±0.0273 (10.4%)	0.773±0.0350 (13.8%)	0.737±0.0151 (16.7%)
5	0.663±0.0344 (25.79%)*	0.647±0.0266 (27.2%)*	0.615±0.0253 (31.43%)*	0.595±0.0179 (32.95%)*
6	0.540±0.057** (38.63%)	0.515±0.0210** (41.8%)	0.395±0.0283** (55.96%)	0.325±0.0266** (63.27%)

*$p<0.05$ vs control, **$p<0.01$ vs control (n=6)

Table: III – Antibacterial-sensitivity testing of 1-6.

Compd. No.	Antibacterial Activity		
	Zone of Inhibition (mm)		
	S. aureus	***E. coli***	***P. aeruginosa***
1	14.3 ± 0.33	18.3 ± 0.33	14.3 ± 0.33
2	20.7 ± 0.67	12 ± 0.00	16.7 ± 0.33
3	21.7 ± 0.67	16 ± 0.00	17.7 ± 0.33
4	16.7 ± 0.67	18 ± 0.00	14.7 ± 0.67
5	22.3 ± 0.67	17.7 ± 0.33	12 ± 0.00
6	23 ± 0.00	18.3 ± 0.33	20.7 ± 0.33
Amoxicillin	26 ± 0.54	25 ± 0.68	26 ± 2.4
DMF	-	-	-

- All the values are expressed as mean ± SEM of triplicates

Table: IV– Antifungal-sensitivity testing of 1-6.

Compd. No.	Antifungal Activity		
	Zone of Inhibition (mm)		
	C. albicans	***A. flavus***	***A. fumigates***
1	10.3 ± 0.33	8 ± 0.00	9 ± 0.00
2	9 ± 0.00	11 ± 0.00	10 ± 0.00
3	10 ± 0.00	11 ± 0.00	8 ± 0.00
4	8 ± 0.00	11.7 ± 0.67	9 ± 0.00
5	10.3 ± 0.33	12 ± 0.00	9.3 ± 0.33
6	14 ± 0.00	13 ± 0.00	9 ± 0.00
Griseofulvin	24± 0.00	25 ± 0.00	23 ± 0.00
DMF	-	-	-

- All the values are expressed as mean ± SEM of triplicates

CONCLUSIONS

After screening the designed compounds for anti-inflammatory and anti-microbial (anti-bacterial and anti-fungal) studies it was found that each compound **1-3** and **4-6** possesses anti-inflammatory activity and anti-microbial activity to certain extent. Among the newly synthesized derivatives, compound **6** have shown significant ($p<0.01$) anti-inflammatory activity and was found to be almost equipotent to indomethacin when tested on rats. The compounds **3** and **5** have also shown significant ($p<0.05$) results. The other tested compounds **1, 2 and 4** have also shown anti-inflammatory activity to certain extent. Anti-microbial (anti-bacterial and anti-fungal) screening revealed that among the newly synthesized derivatives, compound **6** have shown the most significant anti-microbial activity when compared to standard drugs. Compound **5, 3 and 2** were found to have moderate activity while compound **1** and **4** were found to have mild activity among the tested compounds. After comparing the anti-inflammatory activity, anti-microbial activity and structural configuration of compounds **1-3** and **4-6**, it was concluded that the incorporation of chlorine in derived compounds enhances their activity.

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