Synthesis and studies of antimicrobial activities of Indazole, Benzisoxazole and Thione derivatives

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Abstract:

Indazole, benzisoxazole and Thione derivatives has been synthesized derived from (2E, 6E) -2,6 diarylidine cyclohexanone as synthone . This moiety is having exocyclic c=c bond on both side of carbonyl group and usually tend to react with carbonyl reagents such as hydrazine hydrate, phenylhydrazine, hydroxyl amine hydrochloride and thiourea.

Introduction:

Indazole, Benzisoxazole and Thione derivatives plays important role in many physiological process. Their pharmaceutical importance plays attention towards the synthesis of these derivatives derived from (2E, 6E)-2,6 Diarylidine cyclohexanone as a synthone, which in turn synthesize from aldol condensation of cyclohexanone with aromatic aldehyde using 10% sodium hydroxide solution.

Diarylidine cyclohexanone consists of exocyclic double bond adjacent to carbonyl group which is useful for condensation reaction with hydrazine hydrate, phenylhydrazine, hydroxyl amine hydrochloride and thioure .

Experimental:

All the melting points were determined in open capillary tube and maybe uncorrected. The purity of compound was checked by TLC on silical gel coated glass plate. Infrared spectra were monitored and determined in Nujol/KBR plates by using the Bomen 104 ft infrared spectrophotometer. 1H NMR spectra were obtained on Germani 200 mz spectrometer. Elemental analysis was performed on a Heraeus CHNO Rapid analyser.

Experiment No. 1:

Aldol condensation of cyclohexanone with aromatic aldehyde and its dehydration of the product formed.

General procedure:

To a warm solution of cyclohexanone (0.2 M i.e. 19.6 g), benzaldehyde (0.4 M i.e. 42.4 g) in ethanol (50 ml), 10% sodium hydroxide (20 ml) was added. The reaction mixture was stirred until semisolid separated. It was washed with ice cold ethanol, dried and crystallised from ethanol to get yellow coloured shining needles in 80 - 85% yield.

The aldol obtained above was subjected to dehydration using HCL and CH_3COOH mixture in (1:10 ratio) and resulting mixture was refluxed for 15 min, cooled and poured over crushed ice to get orange coloured crystalline solid . It was crystallised from ethanol.

PROPERTIES AND CONSTITUTION OF THE COMPOUND (2) , m. p., 117 - 118°C:

1) TLC studies indicates Rf value = 0.79 in acetone an eluant.
2) The compound is orange coloured crystalline solid m.p. 117 - 118°C.
3) Analytical results indicated the molecular formula of the compound to be $C_{20}H_{18}O$.
4) IR : (IR Plate No. 2) 3040 C = CH aromatic str, 1661.6 cm-1, >C-O str.
5) PMR : (PMR Plate No. 2) □ 1.8 m, 2Hb protons; □ 2.85 m 4Ha protons; □ 7.4- 7.8 m, 10H, ArH and 2H, $=CH_c$ protons.

Analysis:

Found C, 87.02; H, 6.65.
Calculated for $C_{20}H_{18}O$, C, 87.59; H, 6.56.
Based on analytical and spectral data compound was assigned the structure as bis benzylidene cyclohexanone.

Experiment 2

Synthesis of (7E)-7-benzylidene-3-phenyl-3,3a,4,5,6,7-hexahydro-2H indazole (3).

A mixture of (2E, 6E)-2,6-dibenzylidene cyclohexanone (0.02M,1g), hydraxine hydrate(0.02

M, 1 g} in dioxane (15 ml) was heated under reflux for three hours. The resulting mixture was concentrated cooled and poured over crushed ice to get (3) in 70% yield .The product was crystallised by using dioxane as a solvent .

PROPERTIES AND CONSTITUTION OF THE COMPOUND (3) , m. p., 150 - 151°C:

1. Compound is yellow coloured crystalline solid m. p. 150-151°C.
2. TLC studies indicated Rf =0.89 in dioxane as eluant.
3. Elemental analysis indicated the molecular formula as $C_{20}H_{20}N_2$
4. Spectral data is as follows.

IR:[IR Plate No. 3] 3273 cm^{-1} , NH Str.; 3058.60 cm^{-1} aromatic;1600.6 cm^{-1} C=N Str.
PMR : [PMR Plate No.3] 1.59-2.74, m, 6H (3 x CH2); 3.70, s, 1H pyrazole ring proton; 5.57, s, 1H, NH proton; 7.2 - 7.65, m, 12H, 10Ar-H and 2H for Ph-CH proton.

Analysis:

Found; C, 82.98; H, 6.99; N, 9.79.
Calculated for $C_{20}H_{20}N_2$; C, 83.30; H, 6.94; N, 9.72.

Experiment 3

Synthesis of (7E) - 7- benzylidene-2, 3-diphenyl-3, 3a, 4, 5, 6, 7- hexahydro-indazole (4).

A mixture of (2E, 6E)-2, 6-dibenzylidene cyclohexanone (0.01 M. 2.74 g), Phenyl hydrazine (0.01 M, 1.08 g) and dioxane (20ml) was heated under reflux for 4 hr. The resulting mixture was cooled and poured over crushed ice. The product so obtained was filtered to get (4) in 78% yield. The product was crystallised from alcohol, dioxane mixture.

PROPERTIES AND CONSTITUTION OF THE COMPOUND (4) , m. p., 135 - 136°C:

1. Compound is yellow crystalline solid m. p. 135-136°c.
2. TLC studies indicated Rf =0.92 in acetone as eluant.
3. Elemental analysis indicated the molecular formula as $C_{26}H_{24}N_2$
4. Spectral data is as follows.

IR: [IR Plate No. 4] 3022 cm^{-1}, aromatic; 1606.9 cm^{-1}, C=N Str.
PMR: [PMR Plate No. 4] 1.25-3.0, m, 6H, (3 x CH_2); 4.56-4.60, 1H, ring proton; 6.60-7.44, m, 17H, 15Ar-H & 2H for Ar- C=CH.

Analysis:

Found: C, 85.69; H, 6.62; N, 7.52.
Calculated for $C_{26}H_{24}N_2$; C, 85.71; H, 6,59; N, 7.69.
On the basis of these properties the compound (4) was assigned the structure as (7E)-7-benzylidene-2, 3-diphenyl-3, 3a, 4, 5, 6, 7-hexahydro-indazole.

Experiment 4

Synthesis of (7E) - 7-benzylidene - 3 - phenyl - 3, 3a, 4, 5, 6, 7- hexahydro-2, 1-benzisoxazole (5).

A mixture of (2E, 6E)-2, 6-benzylidene cyclohexanone (0.01 M, 2.74 g), aqueous solution of hydroxylamine hydrochloride (0.02 M, 1.4 g) potassium hydroxide, (0.02 M, 1.10 g) in alcohol (20 ml) was heated under reflux for 4 hr. The resulting mixture was concentrated cooled, poured over crushed ice and acidified with dilute acetic acid to get (5) in 79% yield. The product was crystallised from alcohol.

PROPERTIES AND CONSTITUTION OF THE COMPOUND (5), m. p., 165 - 166°C:

1. Compound is pale yellow coloured crystalline solid m. p. 165- 166°c.
2. TLC studies indicated Rf=0.87 in hexane as eluant.
3. Elemental analysis indicated the molecular formula as $C_{20}H_{19}NO$.
4. Spectral data is as follows:

IR: [IR Plate No. 5] 3057.9 cm^{-1} aromatic; 1600.2 cm^{-1}, C=N Str.
PMR: [PMR Plate No. 5] 1.55-2.59, m, 6H, (3 x CH_2); 4.80, s, 1H ring proton; 7.007.40, m, 10H, Ar-H; 9.09-9.29, 2H for Ar-C=CH.
Analysis:
Found: C, 82.98; H, 6.50; N, 5.01.
Calculated for, $C_{20}H_{19}NO$; C, 83.04; H, 6.57; N, 4.84.

Thus, on the basis of all these properties compound was assigned the structure as (7E)-7-benzylidene-3-phenyl-3, 3a, 4, 5, 6, 7-hexahydro-2, 1-benzisoxazole.

Experiment 5

Synthesis of (8E) - 8 - benzylidene - 4 - phenyl - 3, 4, 5, 6, 7, 8- hexahydro quinazoline-2(1H)-thione (6).

A mixture of 2,6-dibenzylidene cyclohexanone (27.4 g ,0.1 mol), thiourea (7.6 g, 0.1 mol) and potassium hydroxide (6.41 g, 0.11 mol) in ethanol (150 ml) was heated under reflux for 3 hr. The reaction mixture was concentrated to half to its volume, diluted with water and then acidified with dil acetic acid. The solid thus obtained was filtered washed with water and crystallised from ethanol to get (6). m.p. 182 - 184°C. in 70% yield.

PROPERTIES AND CONSTITUTIONS OF THE COMPOUND (6), m. p. 182 - 184°c.

1) Compound is a faint yellow coloured crystalline solid, m. p. 182 - 184°C.
2) Its TLC studies showed RF value to be 0.69 in acetone as eluant.
3) Desulphurisation test is +ve :-To a solution of (6) in ethanol was added few drops of NaOH and lead acetate. On heating a black precipitate of lead sulphide is obtained. This indicates the presence of free sulphur.
4) Elemental analysis of the product indicated its molecular formula to be $C_{21}H_{20}N_2S$.
5)The spectral data of the compound is as follows.

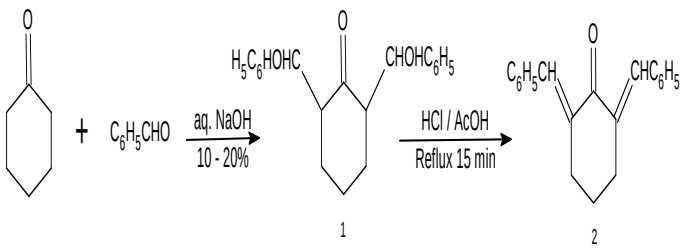
IR : [IR Plate No. 6]3458-3380- NH str, 3056 aromatic C=C-H str., 1219.8 cm^{-1} C=S str. 700 - 750 cm^{-1} aromatic substitution pattern .

PMR [PMR Plate No. 6] □ 1.6 to 2.7, 6H, m (3 x CH_2) of cyclohexanone moiety, □ 4.9, 1H, s, H_a, □ 6.5, 1H, H_e, □ 7- 7.3, 10H, m, Ar-H; □ 7.67 1H, s, (Ar-CH).

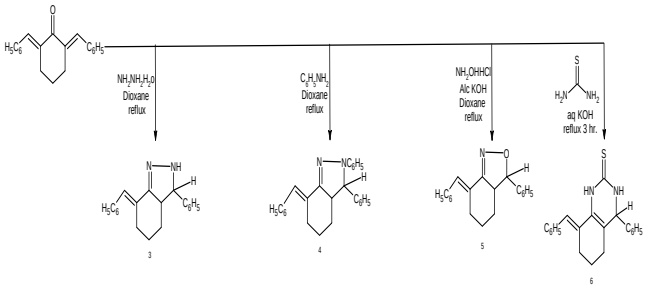
Found : C,76.01; H,5,97; N,8.56; S,9.35

Calculated for: $C_{21}H_{20}N_2S$; C,75.90; H,6.02; N,8.43; S,9,63

Thus, from chemical and spectral properties, the compound was assigned the structure as (8E)-8-benzylidene-4 phenyl-3,4,5,6,7,8-hexahydro quinazoline-2 (1H)-thione.



Scheme



Result and Discussion

The structures of synthesis compound 2 to 6 were assign on the basis of elemental analysis and spectral data and were in good agreement with spectral evidences.

All the synthesized compound possess antibacterial activity against gram +ve and gram -ve bacteria and shows low to moderate activity. Some of them also possess antifungal activity.

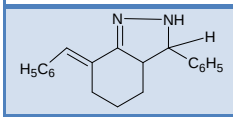
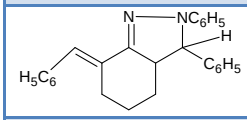
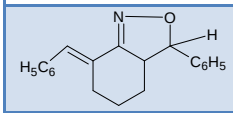
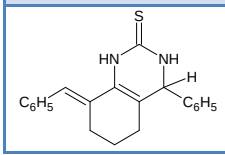
Antibacterial Activity:

The antimicrobial activities of the synthesized heterocyclic compound were tested against the pathogenic bacteria using Cup-plate method and the minimum inhibitory concentration (MICS) where determined using broth macro dilution method. The organism used for both these methods includes gram positive S. aureus, Bacillus Cerus and gram negative bacteria E.Coli, S.Typhi, Psuedomenous Aeruginosa, P. Valgoris and also tested for antifungal activity against A. niger, fujarim SP by cup plate method while MIC values were determined using

Broth macro dilution method.

In the present studies the activity of test compounds were screened as poor with inhibition zone less than 14 mm moderate with inhibition zone 14 to 18 mm and higher with inhibition zone size higher than 18 mm.

All the results are recorded in the table.

Structure	E.Coli	S.Typhi Salmonella	Psuedomenous Aeruginosa	Bacillus Cerus	S. Aureus	P. Valgoris	A. Niger	Fujarim sp
	14	15	00	00	16	14	15	14
	14	14	09	00	16	13	18	20
	14	12	10	00	12	16	15	14
	16	12	00	00	10	12	20	18

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