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STUDY AND SYNTHESIS OF SOME ORGANIC SPIRO DERIVATIVES USING SCHIFF BASE REACTION

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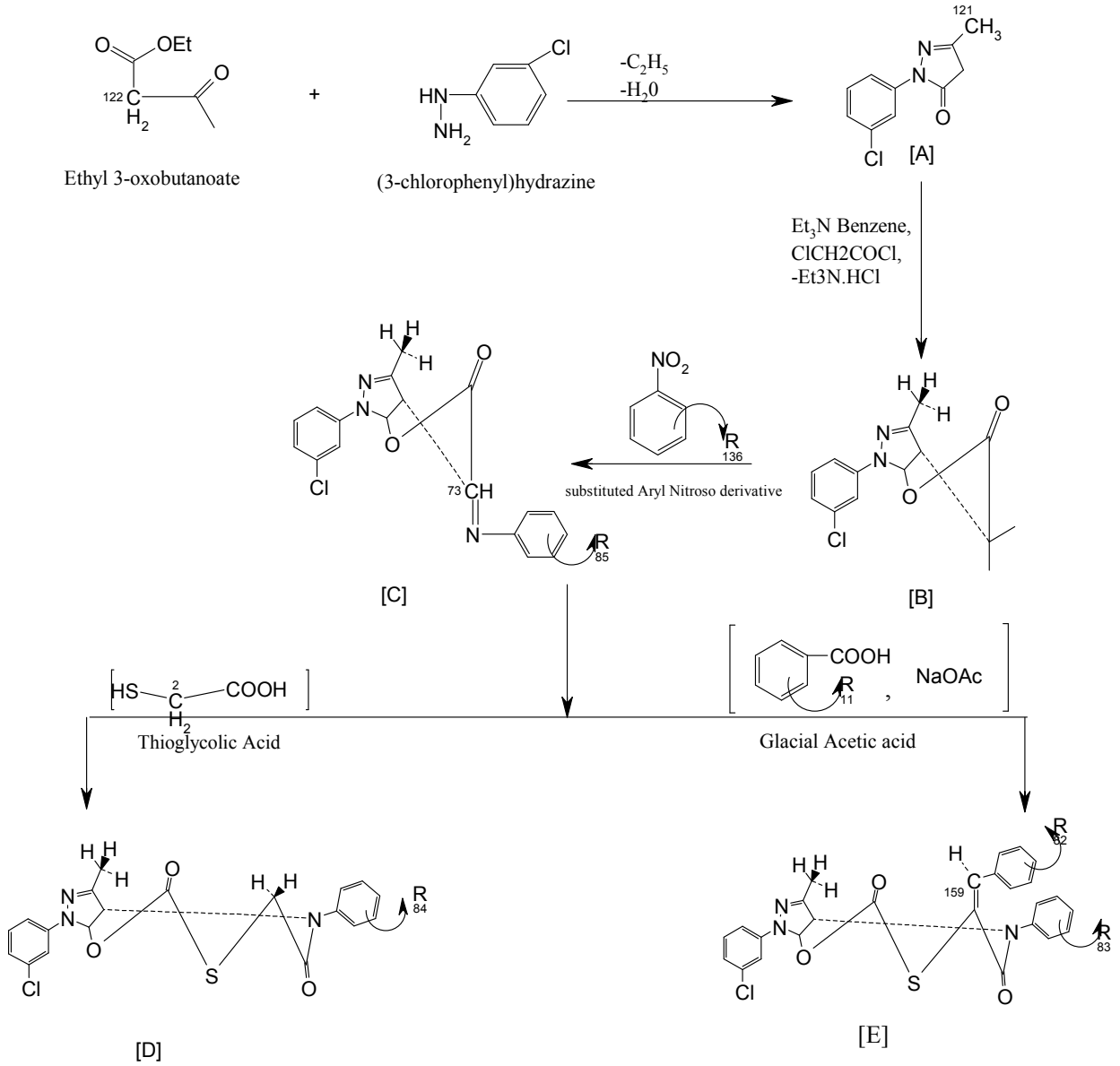
ABSTRACT:
When two cyclized ring systems are joined in such a manner, where a single carbon atom is at the junction of two ring system are called spiro compounds.The organic spiro compound possess good biological activities, anticancer as well as antitumor activity.It is known that the reaction of nitroso compound with active methylene groups produces iminoderivative, the condensation, known as the 'Ehrlich-Sachs reaction', is catalyzed by based such as sodium hydroxide, K_2CO_3 & piperidine. Pyrazole and pyrazolone derivatives are known to possess some important biological activities such as antiarthritic, antihypertensive, antibalterial and antifungal, artihistaminic hypoglycemic etc.Higher activity of pyrazole ring containing compound and schiff base derivatives promoted to synthesize pyrazoline compounds incorporating fused furanose moiety containing reactive methylene group derivative which was then made react with several aryl micros compounds to synthesize schiff base derivatives and studied their antimicrobial activities.

KEYWORDS: Anticancer, schiffbase derivatives, pyrazole and pyrazoline derivatives antimicrobial, organic spiro derivatives, antitumor.

REACTION SCHEME:



In this reaction scheme,followings are included: -

[**A**] 1-(3'-chloro) phenyl – 3-methyl – 5 – pyrazolone , [**B**] 1-[3'- chlorophenyl] – methyl – pyrazolo – (4,5-b)-furan -2-one, [**C**] 1-[3'-chlorophenyl] -3- methhyl – pyrazolo – (4,5-b) – 3 phenyl imino furan-2 – one, [**D**] 1-[3'-chlorophenyl] – 3 methyl – pyrazolo (4,5-b) furan – 3 – spiro (3" – N –aryl – 4" – oxothiazoldine) – 2 – one, [**E**] 1-[3'-chlorophenyl] -3- methhyl – pyrazolo – (4,5-b) – furan-3-spiro-[3"-N- phenyl-5"-arylidine-4"-oxo-thiazolidine]-2-one

1. Preparation of 1-(3'-chloro) phenyl – 3-methyl – 5 – pyrazolone

Mixture of ethyl acetoacetate (13 gm 0.1 mole) and 3-chloro phenyl hydrazine (14.35g, 0.1 mole) in large evaporating dish was heated on boiling water bath in fume cupboard for 2 hours with occasional string with a glass road. After the completion of reaction, a heavy reddish syrup was obtained which was cooled and 100 ml of ether was added to it and the mixture was solidified with in 15 minutes and was filtered at pump and washed with ether. Re-crystallized it from eq. amount of ethanol and water.

yield :- 80%, MP : 128 – 131^{0}c.

2. Synthesis of 1-[3'- chlorophenyl] – methyl – pyrazolo – (4,5-b)-furan -2-one

A mixture of 1 [3-chloro phenyl] -3- methyl pyrazole 5 one (0.01m) and monochloroacetyl chloride (0.01M) in benzene 30 ml with triethylamine (2.6 ml) was refluxed for 8 hrs, the reaction mixture was filtered, concentrated and allowed to cool at room temperature.

yield 78%, M.P 152^{0}c.

3. Synthesis of 1-[3'-chlorophenyl] -3- methyl – pyrazolo – (4,5-b) – 3 phenyl imino furan – 2 – one

A mixture of 1-[3' chloro phenyl -(4,5-b) – furan-5-one 2.48 (0.01m) and nitroso compound [nitroso benzene 1.07g, (0.01 mole)] in butanol 20ml containing sodium methoxide (22%) was refluxed for 10-14 hrs. The hot reaction mixture was filtered, concentrated and allowed to cool at room temperature for 3 days.

yield obtained was 2.76 gm (82%) M.P = 143^{0}C.

Formula	%	C	H	N
$C_{18}OH_{12}N_3O_2Cl$	Found	64.05	3.58	12.47
	Required	64.02	3.55	12.43

4. Synthesis of 1-[3'-chlorophenyl] – 3 methyl – pyrazolo (4,5-b)-furan – 3 – spiro (3" – N – phenyl – 4" – oxothiazoldine) – 2 – one.

A mixture of imino derivatives 3.37g (0.01m) and mercapto acetic acid (0.01M) in drybenzene was refluxed for 12-15 hrs the hot reaction mixture was filtered and concentrated. The product was re-crystallized from DMF and water.

M.P :- 192^{0}C, Yield - 2.46 g (60%)

Formula	%	C	H	N
$C_2OH_{14}N_3O_3cl_5$	Found	58.37	3.43	10.25
	Required	58.34	3.40	10.20

5. Synthesis of 1-[3'-chlorophenyl] -3- methyl – pyrazolo – (4,5-b) – furan-3-spiro-[3"-N-phenyl-5"-Benzylidine-4"-oxo-thiazolidine]-2-one

To a solution of Benzaldehyde (1.06g,0.01 M) and 4-thiazolidinone derivative (1.11 g, 0.01 M) in glacial acetic acid (25 ml) , anhydrous sodium acetate (1.23 g, 0.015 M) was added, the reaction mixture was refluxed for 6 hours, filter out the reaction mass and re-crystallized from DMSO.

M.P :- 193^{0}C, Yield - 3.64 g (73%)

Formula	%	C	H	N
$C_{27}OH_{18}N_3O_3cl_5$	Found	64.92	3.65	8.44
	Required	64.88	3.60	8.40

CHARACTERIZATION OF THE COMPOUNDS:

The synthesized compounds were characterized by preliminary laboratory techniques such as melting point, boiling point etc. and their structures will be confirmed by FTIR, Mass and NMR spectral data.

Screening of Angiotensin Converting Enzyme inhibitory activity:

***In vitro* screening of angiotensin converting enzyme inhibitory activity:**

An *invitro* system used to screen potential angiotensin – converting – enzyme inhibitors. Fluorescence generated by an artificial substrate in the presence or absence of inhibitor was measured to detect inhibitory activity.

Enzyme preparation study:

Lung tissue from 10 rats was diced and homogenized in a blender with 3 pulses of 15 s each. The homogenate was centrifuged at 5 000 *g* for 10 min. The pellet was discarded, the supernatant was dialyzed against three 1 liter changes of 10 mM potassium phosphate buffer, pH 8.3 overnight in the cold and then it was centrifuged at 40,000 *g* for 20 min. The pellet was discarded, 390 mg $(NH_4)_2SO_4$ was added for each ml of supernatant. This gave 60% saturation. The solution was stirred on ice for 15 min. The pellet formed was dissolved in 15 ml potassium phosphate buffer, pH 8.3 and dialyzed against the same buffer overnight in the cold with three 1 liter changes. Some protein precipitated during dialysis. The suspension was centrifuged at 40,000 *g* for 20 min and the supernatant was discarded. The final solubilized enzyme preparation liquoted and stored at –20 °C at least 6 months.

Enzyme inhibition Studies :

Enzyme activity was measured with a Perkin Elmer LS – 5 Fluorescence Spectrophotometer or equivalent at an excitation wave length of 357 nm and an emission wave length of 424 nm. 50 µL vehicle or inhibitor solution and 40 µL enzyme was preincubated for 5min, then 410 µL substrate working solution was added. Samples were mixed by drawing fluid back up into the pipette and by pipetting into the cuvette.

Individual fluorescence slope was measured and % inhibition is calculated as follows :

$$\% \text{ inhibition} = 100 - \frac{\text{Slope in presence of inhibitor}}{\text{Control slope}} * 100$$

Inhibitor concentrations on either side of IC_{50} should be tested to generate a dose – response curve. IC_{50} is calculated using Litchfield – Wilcoxon log – probit analysis.

Anticancer screening data of tested compounds:

Comp No.	60 cell line assay in one dose at 10-5 concentration			
	Mean growth %	Range of growth %	Most sensitive cell line	Growth of most sensitive cell line %
1	100.78	-20.59 to 40.60	CNS cancer	-20.59
2	98.35	-29.69 to 55.77	Renal cancer	-29.69
3	96.81	-28.61 to 47.91	Renal cancer	-28.61
4	84.09	-65.06 to 98.06	Ovarian cancer	-65.06
5	104.54	-17.68 to 44.00	Renal cancer	-17.68

CONCLUSIONS

In the present paper five compounds were tested and most of them displayed antitumor activity on renal cancer,CNS cancer cell and ovarian cancer cell lines. The most efficient anticancer compound (**1**) was found to be active with selective influence on ovarian cancer cell lines.The obtained results prove the necessity for further investigations to clarify the features underlying the antitumor potential of tested compounds.

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