FORM – 2
THE PATENTS ACT, 1970
(39 of 1970)
&
THE PATENTS RULES, 2003

PROVISIONAL SPECIFICATION

(See section 10 and rule 13)

CONJUGATES OF N-BENZYLPYRROLE OR N-BENZOYLPYRROLE WITH INDOLINONE AS HIGHLY EFFECTIVE ANTI-CANCER AGENTS

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of

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THE FOLLOWING SPECIFICATION PARTICULARLY DESCRIBES THE INVENTION AND THE MANNER IN WHICH IT IS TO BE PERFORMED

FIELD OF THE INVENTION

The present invention relates to conjugates of N-benzylpyrrole or N-benzoylpyrrole and indolinone which have anticancer activity, methods of synthesizing such compounds and compositions thereof.

BACKGROUND

Apart from the cardiovascular diseases, cancer comes out to be a leading cause of global mortality and is responsible for approximately 21% annual deaths worldwide. Population growth and aging along with increasingly adoption of cancer associated lifestyle are the primary causes for increasing burden of cancer especially in economically developing countries.

Chemotherapy is a widely used treatment for majority of cancers and various chemotherapeutic drugs (taxol, vinblastine, vincristine, etoposide, camptothecin, mitoxantrone, 5-fluorouracil, cisplatin, etc.) are in use. Nonetheless, most of these drugs are associated with severe side effects including anaphylaxis, mucosiuis, alopecia, leucopenia, neutropenia, neurological oxicities, gastrointestinal, renal, hepatic, and cardiac effects etc (Brighton, D.; Wood, M. In *The Royal Marsden Hospital Handbook of Cancer Chemotherapy, Elsevier Churchill Livingstone, 2005*). Interactions with the undesired targets, non-selectivity, lack of effectiveness and

cancer resistance to drug treatment are responsible causes for the failure of most of anticancer drugs.

Almost all the currently FDA approved anticancer drugs such as Paclitaxel, 5-flurouracil, Methotrexate, Cisplatin, 6-Mercaptopurin, etc. are suffering from severe side effects (www.nlm.nih.gov/medlineplus).

So there is an urgent need to develop new chemical entities for an effective, safe and economical treatment of cancer.

Nitrogen containing heterocycles are always of immense importance because they are ubiquitous in nature as well as found as backbone skeleton of many pharmaceutical compounds. Pyrrole and indolinone nucleus holds vital place in medicinal chemistry and serves as a template of the development of various anticancer, anti-inflammatory, antibiotic, antimalarial agents etc.

OBJECTIVES OF THE INVENTION:

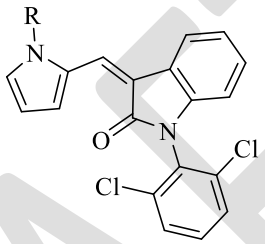
An object of the present invention is to provide compounds that exhibit appreciable anti-cancer activity against cancer, especially breast cancer, renal cancer, ovarian cancer and prostate cancer. Desirably, the compounds exhibit low toxicity.

Another object of the invention is to provide method of preparations of compounds that exhibit appreciable anti-cancer activity against cancer.

Another object of the invention is to provide compositions of the compound that exhibit appreciable anti-cancer activity against cancer.

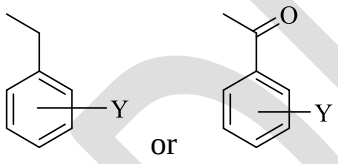
SUMMARY OF THE INVENTION:

The present invention relates to conjugates of benzyl or benzoyl substituted pyrrole and indolinone moieties of Formula (I) which have appreciable anticancer activity.



(I)

wherein R is



or

Pyrrole and indolinone conjugates have been synthesized and evaluated for anticancer activity.

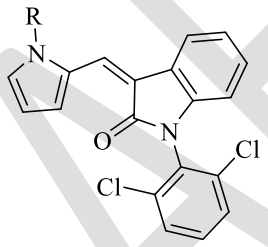
These compounds (i) exhibit growth inhibitory activity against various human cancer cell lines, (ii) show remarkable growth inhibition activity over selected cancer cell lines. (iii) have the 50% inhibitory conc. of the compounds in nM range.

Another embodiment of the invention relates to method of synthesizing compounds of Formula (I).

Another embodiment of the invention relates to compositions comprising compounds of Formula (I) along with pharmaceutically acceptable excipients.

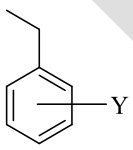
DETAILED DESCRIPTION OF THE INVENTION:

The present invention relates to conjugates of benzyl or benzoyl substituted pyrrole and indolinone moieties of Formula (I) which have anticancer activity.

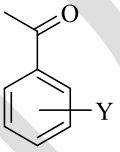


(I)

wherein R is



or

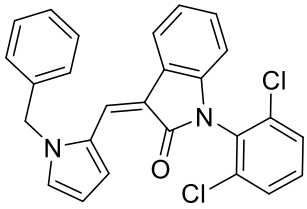
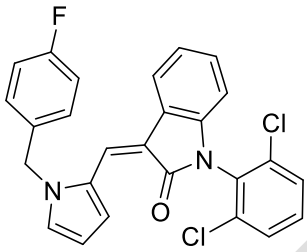
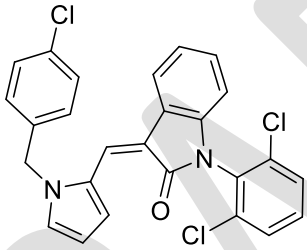
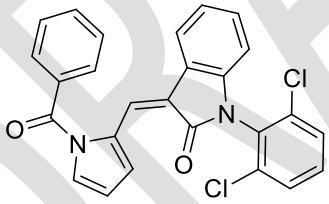
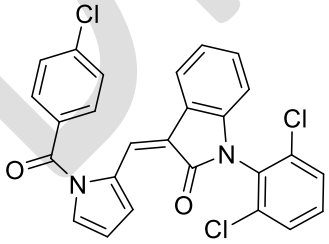


wherein Y is hydrogen or halogen.

Preferably, Y is attached at the para-position of the benzyl or benzoyl ring.

More preferably, the compounds are selected from the below mentioned compounds:

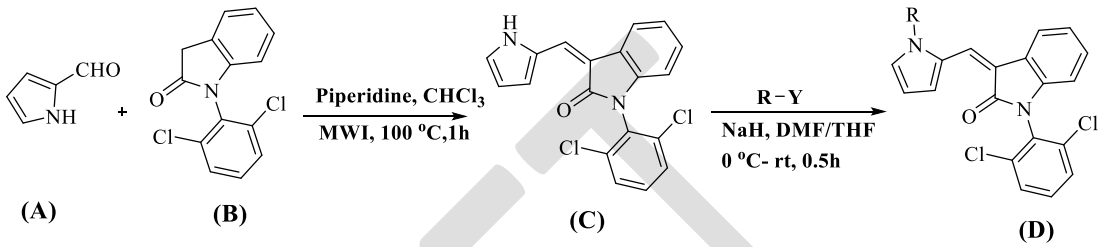
Compound Code	Structure	IUPAC name

1		3-(1-Benzyl-1*H*-pyrrol-2-ylmethylene)-1-(2,6-dichloro-phenyl)-1,3-dihydro-indol-2-one
2		1-(2,6-Dichlorophenyl)-3-[1-(4-flurobenzyl)-1*H*-pyrrol-2-ylmethylene]-1,3-dihydroindol-2-one
3		3-[1-(4-Chlorobenzyl)-1*H*-pyrrol-2-ylmethylene]-1-(2,6-dichlorophenyl)-1,3-dihydroindol-2-one
4		3-(1-Benzoyl-1*H*-pyrrol-2-ylmethylene)-1-(2,6-dichlorophenyl)-1,3-dihydroindol-2-one
5		3-[1-(4-Chlorobenzoyl)-1*H*-pyrrol-2-ylmethylene]-1-(2,6-dichlorophenyl)-1,3-dihydroindol-2-one

Methods of synthesis of compounds of Formula (1-5)

Method I

The method comprises following steps:



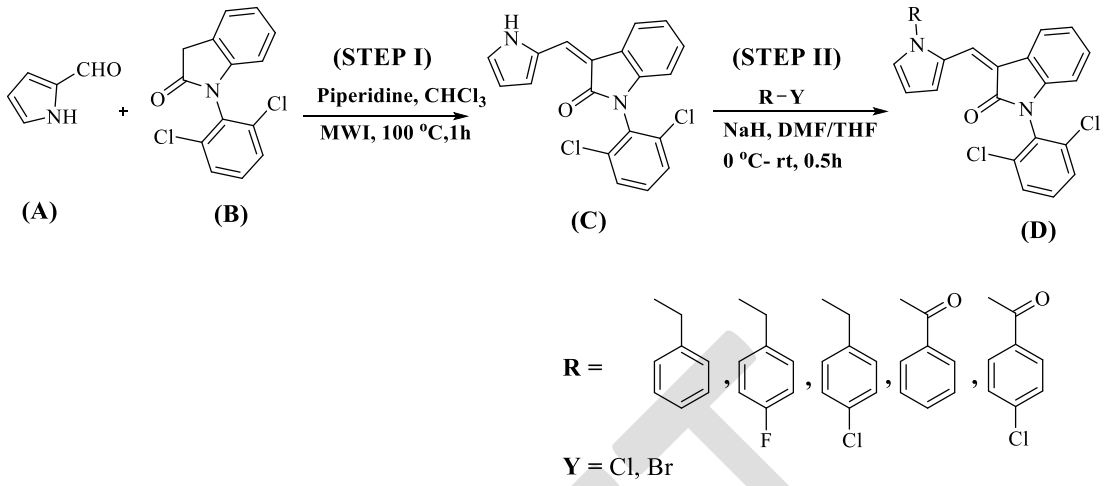
(A) **(B)** **(C)** **(D)**

wherein

R is selected from unsubstituted or halogen substituted benzyl group or unsubstituted or halogen substituted benzoyl group; and

Y is halogen.

Compounds of the present invention were prepared by following Method I as:



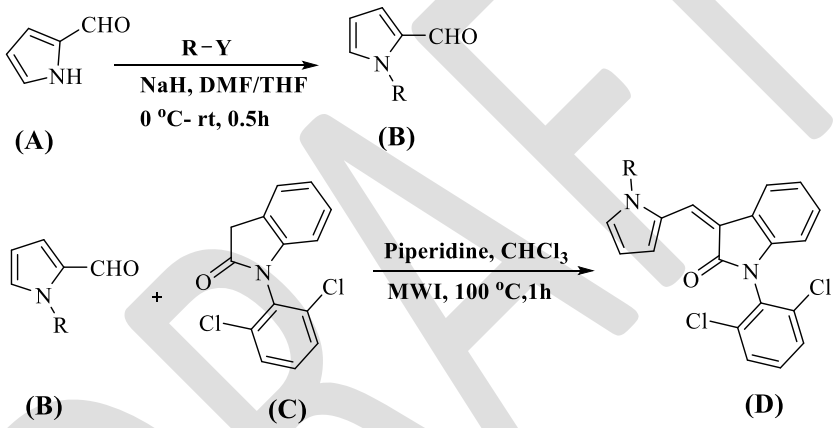
Step I. Compounds (A), (B) and aryl halides (R-Y) are commercially available. Compound (C) was prepared by reacting 1 mmol each of compounds (A) and (B) in chloroform in the presence of catalytic amount of piperidine, then the reaction mixture was subjected to microwave irradiations for 1 hr. After the completion of reaction, water was added to the reaction mixture and it was extracted with chloroform. Then the organic layer was dried over sodium sulfate and distilled off under vacuum to give crude product. Crude product (C) was purified by recrystallization from chloroform: methanol (3:7).

Step II. For the synthesis of compound (D), 1 mmol of compound (C) was first treated with NaH (1.5 mmol) in polar solvent such as tetrahydrofuran or dimethylformamide at 0 ^{o}C for 10 mins, then corresponding aryl halide (R-Y, 1.5 mmol) was added. After 15 min the reaction was shifted to room temperature for another 15 mins, the progress of reaction was monitored by TLC. After the

completion of reaction, water was added to the reaction mixture and it was extracted with ethyl acetate. The organic layer was then dried over sodium sulphate and distilled off, the crude product was then purified by column chromatography using ethyl acetate and hexane as eluent.

Method II

The method comprises following steps:

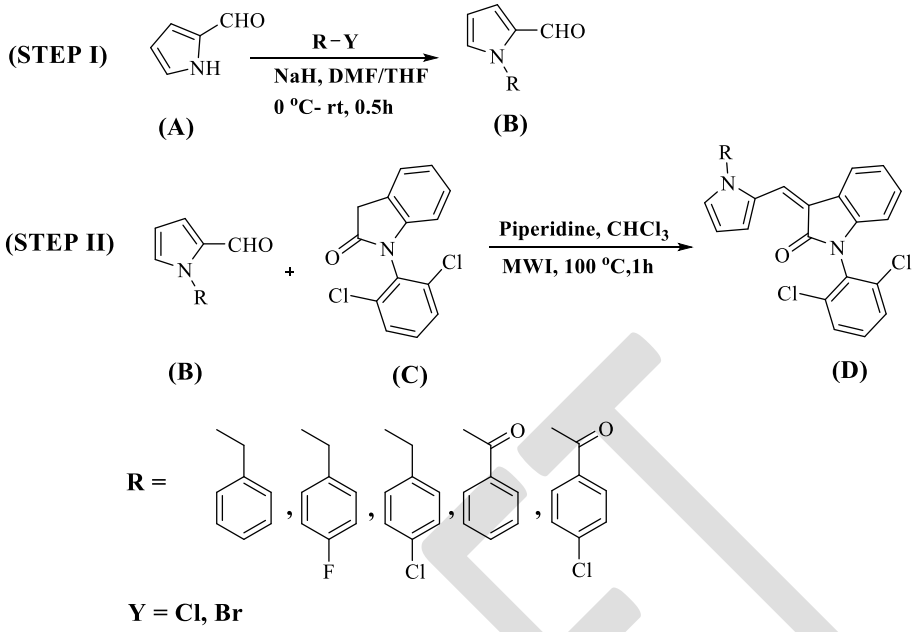


wherein

R is selected from unsubstituted or halogen substituted benzyl group or unsubstituted or halogen substituted benzoyl group; and

Y is halogen.

Compounds of the present invention were prepared by following Method I as follows:



Step I. To synthesize compound (B), 1 mmol of compound (A) was first treated with NaH (1.5 mmol) in polar solvent such as tetrahydrofuran or dimethylformamide at 0 ^{o}C for 10 mins, then corresponding aryl halide (R-Y, 1.5 mmol) was added. After 15 min, the reaction was shifted to room temperature for another 15 mins, the progress of reaction was monitored by TLC. After the completion of reaction, water was added to the reaction mixture and it was extracted with ethyl acetate. Organic layer was then dried over sodium sulphate and distilled off, the crude product was purified by column chromatography (ethyl acetate: henaxe as eluent).

Step II. Compound (D) was prepared by microwave irradiating a solution of 1 mmol each of compounds (B) and (C) in chloroform in the presence of catalytic amount of piperidine for 1 h. After the completion of reaction, water was added to the reaction mixture and it was extracted with chloroform, the organic layer was dried over

sodium sulfate and distilled off under vacuum to get crude product. Crude product (C) was purified by column chromatography using ethyl acetate and hexane as eluent.

Compositions

Compositions may be prepared by admixing compounds of Formula (I) with pharmaceutically acceptable excipients.

Pharmacological activity

The tumor growth inhibitory activities of compound 1 were investigated on a 60 cell line panel of human cancer cells using the method provided in Holbeck, S. L.; Collins, J. M.; Doroshow, J. H. *Mol. Cancer Ther.* **2010**, 9, 1451.

The results are shown in Table 1.

Table 1. GI_{50} (μM), TGI (μM) and LC_{50} (μM) for compounds **1.**

	Compound 1			**Cisplatin**			**5-Fluorouracil**			**Bleomycin**			**Etoposide**		
	GI_{50}	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**
Leukemia															
RPMI-8226	1.28	>100	>100	2.51	2.51	2.51	0.05	50.1	100	15.8	25.1	25.1	1.00	1.00	1.00
Non-Small cell Lung cancer															
EKVX	0.41	7.94	>100	2.51	2.51	2.51	63.0	100	100	15.8	25.1	25.1	1.00	1.00	1.00
HOP-92	0.97	17.7	>100	1.99	2.51	2.51	79.4	100	100	0.07	2.51	25.1	1.00	1.00	1.00
Colon cancer															
COLO 205	1.04	9.33	>100	199	2.51	2.51	0.15	63.0	100	5.01	25.1	25.1	1.00	1.00	1.00
HCC-2998	0.87	>100	>100	2.51	2.51	2.51	0.05	39.8	100	10.0	19.9	25.1	1.00	1.00	1.00
HCT-15	0.37	>100	>100	2.51	2.51	2.51	0.1	50.1	100	3.98	25.1	25.1	1.00	1.00	1.00
KM 12	0.38	>100	>100	2.51	2.51	2.51	0.19	39.8	100	15.8	25.1	25.1	1.00	1.00	1.00
Ovarian cancer															
IGROV1	0.54	>100	>100	1.99	2.51	2.51	1.25	31.6	100	2.51	25.1	25.1	1.00	1.00	1.00
OVCAR-3	0.74	>100	>100	2.51	2.51	2.51	0.01	0.31	50.1	5.01	19.9	25.1	1.00	1.00	1.00

OVCAR-4	0.12	>100	>100	2.51	2.51	2.51	3.98	79.4	100	3.98	19.9	25.1	1.00	1.00	1.00
Renal cancer															
TK-10	0.64	>100	>100	2.51	2.51	2.51	1.25	79.4	100	7.94	19.9	25.1	1.00	1.00	1.00
UO-31	3.16	>100	>100	0.15	1.00	2.51	1.58	50.1	100	0.63	7.94	25.1	1.00	1.00	1.00
Breast cancer															
MCF7	0.03	>100	>100	2.51	2.51	2.51	0.07	50.1	100	1.99	25.1	25.1	---	---	---
T-47D	0.21	1.86	>100	1.25	2.51	2.51	7.94	50.1	100	6.3	25.1	25.1	---	---	---
MDA-MB-468	0.06	0.28	1.38	2.51	2.51	2.51	6.3	39.8	100	25.1	25.1	25.1	---	---	---

GI_{50}- 50% growth inhibitory concentration, TGI- total tumor growth inhibitory concentration, LC_{50}- 50% lethal concentration.

The results demonstrate that the tested compound exhibits appreciable anti-cancer activity having GI_{50} in the nM range against leukemia, non-small cell lung cancer, colon cancer, breast cancer, renal cancer and ovarian cancer. The compounds exhibit low toxicity i.e. conc. >100 μM.

Comparative Examples

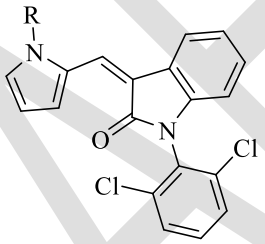
When compared the anticancer data of compound **1** with those commercially available drugs, it was found that compound **1** has better GI_{50} values than approved drugs over the breast cancer and ovarian cancer (Table 1) (https://dtp.cancer.gov/dtpstandard/cancerscreeningdata/index.jsp).

The tumor growth inhibitory activities of compounds were investigated on a 60 cell line panel of human cancer cells. Detailed results of these investigations are given in

terms of GI_{50} (50% growth inhibitory concentration), TGI (total tumor growth inhibitory concentration), LC_{50} (50% lethal concentration), over all the cancer cell lines (Holbeck, S. L.; Collins, J. M.; Doroshow, J. H. *Mol. Cancer Ther.* **2010**, 9, 1451).

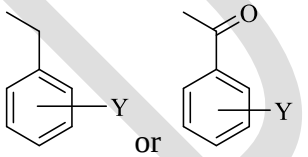
We Claim

1. A compound of formula (I)



(I)

wherein R is



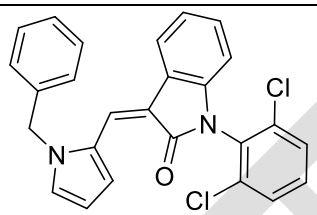
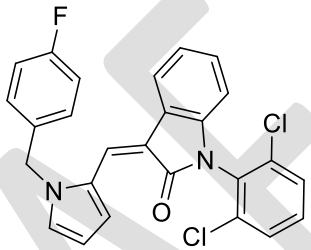
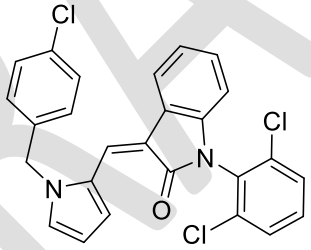
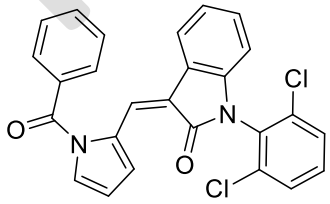
or

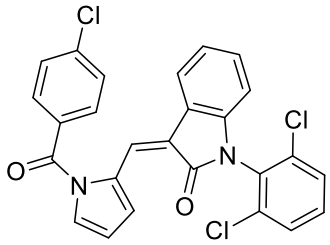
and

wherein Y is hydrogen or halogen.

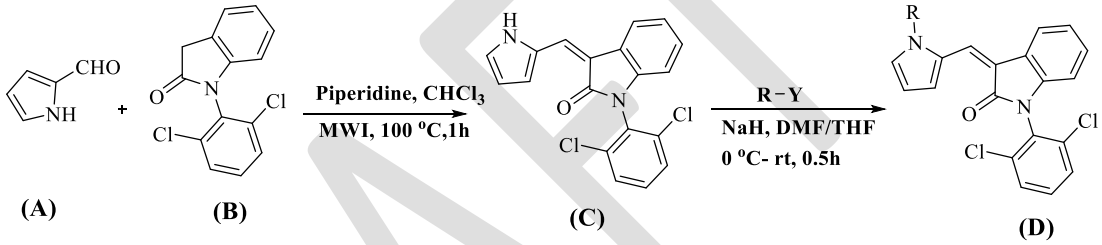
2. The compound as claimed in claim 1, wherein Y is attached at the para position of the benzyl or benzoyl ring.

3. The compound as claimed in claim 1, wherein the compound is selected from

Compound Code	Structure	IUPAC name
1		3-(1-Benzyl-1*H*-pyrrol-2-ylmethylene)-1-(2,6-dichloro-phenyl)-1,3-dihydro-indol-2-one
2		1-(2,6-Dichlorophenyl)-3-[1-(4-flurobenzyl)-1*H*-pyrrol-2-ylmethylene]-1,3-dihydroindol-2-one
3		3-[1-(4-Chlorobenzyl)-1*H*-pyrrol-2-ylmethylene]-1-(2,6-dichlorophenyl)-1,3-dihydroindol-2-one
4		3-(1-Benzoyl-1*H*-pyrrol-2-ylmethylene)-1-(2,6-dichlorophenyl)-1,3-dihydroindol-2-one

5		3-[1-(4-Chlorobenzoyl)-1*H*-pyrrol-2-ylmethylene]-1-(2,6-dichlorophenyl)-1,3-dihydroindol-2-one

4. A process for preparation of compound as claimed in claim 1, comprising following steps:



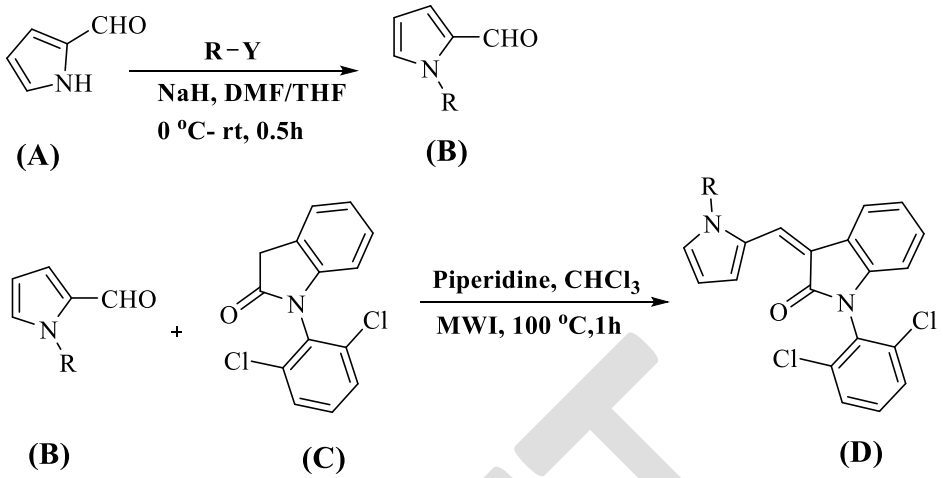
(A) **(B)** **(C)** **(D)**

wherein

R is selected from unsubstituted or halogen substituted benzyl group or unsubstituted or halogen substituted benzoyl group; and

Y is halogen.

5. A process for preparation of compound as claimed in claim 1, comprising following steps:



wherein

R is selected from unsubstituted or halogen substituted benzyl group or unsubstituted or halogen substituted benzoyl group; and

Y is halogen.

6. A composition comprising the compound as claimed in claim 1 and pharmaceutically acceptable excipients.