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Efficient iodine catalyzed three components domino reaction for the synthesis of 1-((phenylthio)(phenyl)methyl)pyrrolidin-2-one derivatives possessing anticancer activities†

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A simple and efficient three components domino reaction of γ-butyrolactam (2-pyrrolidinone), aromatic aldehyde and substituted thiophenol catalyzed by elemental iodine resulted in the formation of 1-((phenylthio)(phenyl)methyl)pyrrolidin-2-one derivatives. The stability of the synthesized analogues was evaluated in stimulated gastric fluid (SGF) and bovine serum albumin (BSA). *In vitro* anticancer activity was investigated in the low micromolar range and a few analogues were found to possess good activity. This current protocol provides several advantages like shorter reaction time, excellent yield and convenient work-up.

It is well-known that 5-membered nitrogen-containing heterocycles are of great biological and pharmacological interest.[1] The γ-butyrolactam ring is implanted in numerous biological compounds as a subunit structure[2] and in several reactions like asymmetric synthesis enantiomerically pure pyrrolidones can also act as chiral auxiliaries.[3]

In the past few years significant importance has been attached to combinatorial synthesis and it has generated considerable interest in the domino reaction,[4] in which several reactions are emerging as useful tools for the formation of carbon–carbon and carbon–heteroatom bonds in synthetic chemistry to create fascinating and novel drug like scaffolds.[5]

In the search for unique therapeutic scaffolds, several research groups have established the individuality of the domino reaction as a powerful tool for the preparation of such molecules, which is the most challenging objective in modern organic synthesis.[6] Thus, in recent times in organic chemistry, the improvement of the new multi-component domino reactions approach was believed to possess some of the green chemistry principles and thus they were accepted as green chemistry methods.[7]

Elemental iodine is emerging as an effective Lewis acid catalyst which has been revealed to be a powerful catalyst for several organic transformations and enhances the utility in organic synthesis.[8] In several combinatorial syntheses, elemental iodine is used as a catalyst and affords numerous advantages like lower reaction time, low cost, excellent yield, convenient work-up and use of simple precursors to synthesize complex molecules.[9]

For the first time we are presenting the synthesis of 1-((phenylthio)(phenyl)methyl)pyrrolidin-2-one derivatives (**4**) *via* a domino reaction of γ-butyrolactam, aromatic aldehyde and substituted thiophenol using iodine as a catalyst and a few of the analogues are found to have good anticancer activity.

In our initial study, we examined the optimized reaction condition to evaluate the efficiency of catalyst for the reaction among γ-butyrolactam, benzaldehyde and 4-bromothiophenol under various conditions. In our model reaction several Lewis acids such as $ZnCl_2$, $AlCl_3$, $HgCl_2$ and CAN were screened and we optimized the reaction condition.

As shown in Table 1, when the reaction was performed without catalyst and with other Lewis acids like $ZnCl_2$, $AlCl_3$, $HgCl_2$ and CAN the reaction did not proceed.

It was remarkable to note that molecular iodine played a significant role in our domino reaction. Thus to investigate the molar percentage of the catalyst required to produce excellent yield, we carried out the reaction with 1 mol% and increased this up to 25 mol% of catalyst. A high yield was observed when using 15 mol% of iodine as the catalyst whereas the use of increased quantities of catalyst did not further improve the yield significantly, and we carried out all the reactions with 15 mol%.

The solvent effect was the next factor considered for better yield. Thus experiments were carried out in various solvents such as polar protic, aprotic and nonpolar solvents using 15 mol% of iodine as the catalyst for all the reactions (Table 1, entry 12–21).

As shown in Table 1, we obtained better yields with polar aprotic solvents such as acetonitrile, dichloromethane (DCM) and tetrahydrofuran (THF) than with polar protic solvents like ethanol, *n*-butanol and isopropyl alcohol (IPA) and nonpolar

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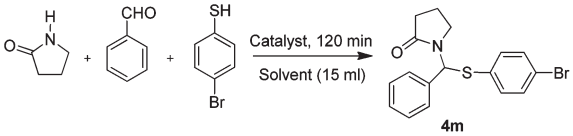
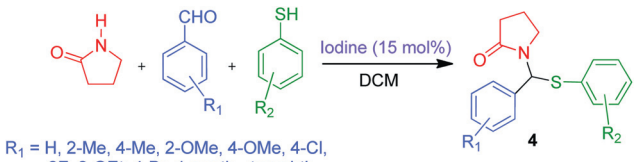
Table 1 Screening of catalyst and solvent effect on the domino reaction of γ-butyrolactam, benzaldehyde and 4-bromothiophenol[a]



Entry	Catalyst (mol%)	Solvent (mL)	Yield[b] (%)
1	None	DCM	—
2	$ZnCl_2$ (25)	DCM	—
3	$AlCl_3$ (25)	DCM	—
4	$HgCl_2$ (25)	DCM	—
5	CAN (25)	DCM	—
6	Iodine (25)	DCM	89
7	Iodine (20)	DCM	89
8	Iodine (15)	DCM	89
9	Iodine (10)	DCM	85
10	Iodine (5)	DCM	79
11	Iodine (1)	DCM	54
12	Iodine (15)	None	—
13	Iodine (15)	CH_3CN	63
14	Iodine (15)	DCM	89
15	Iodine (15)	THF	65
16	Iodine (15)	Ethanol	51
17	Iodine (15)	*n*-Butanol	16
18	Iodine (15)	IPA	12
19	Iodine (15)	Hexane	11
20	Iodine (15)	Benzene	25
21	Iodine (15)	Pentane	28

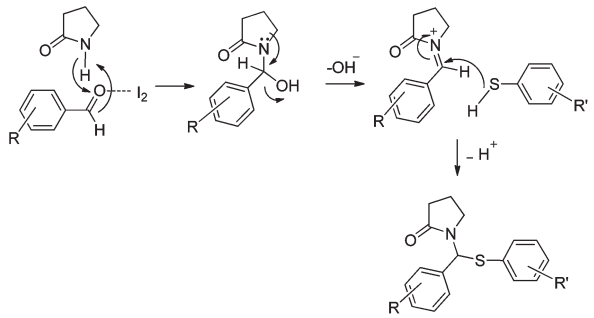
[a] Reaction conditions: γ-butyrolactam (10 mmol), benzaldehyde (10 mmol) and 4-bromothiophenol (10 mmol) at room temperature (25 °C). [b] Isolated yield.

Table 2 Domino reaction of γ-butyrolactam, aromatic aldehyde and substituted thiophenol using iodine as a catalyst leading to the formation of product (**4**)



Entry	Benzaldehyde	Thiophenol	Product	Yield[a] (%)
1	Parent	4-OMe	**4a**	58
2	2-Me	4-OMe	**4b**	62
3	4-Me	4-OMe	**4c**	69
4	2-OMe	4-OMe	**4d**	45
5	4-OMe	4-OMe	**4e**	71
6	4-Cl	4-OMe	**4f**	78
7	2-F	4-OMe	**4g**	89
8	2-OEt	4-OMe	**4h**	65
9	4-Br	4-OMe	**4i**	80
10	1-Naphth-	4-OMe	**4j**	83
11	Terephth-	4-OMe	**4k**	80
12	4-Benzyloxy	4-OMe	**4l**	75
13	Parent	4-Br	**4m**	89
14	4-Cl	4-Br	**4n**	95
15	2-Br	4-Br	**4o**	89
16	4-Br	4-Br	**4p**	92

[a] Isolated yield.



Scheme 1 A possible mechanism for the formation of product (**4**).

Scheme 2 Reaction of γ-butyrolactam and benzaldehyde.

solvents like hexane, benzene and pentane. In the absence of solvent no desired product was obtained and in the case of DCM maximum yield was obtained. Hence, the optimal solvent for these reaction transformations was DCM.

This is in consonance with our proposed mechanism, the presence of EDG in the benzene ring of benzaldehyde made the oxygen form a bond with hydrogen from pyrrolidones very effectively and helped the catalysis of the reaction with iodine in the initial step. Both will be retarded by EDG in the benzene ring of benzaldehydes. In the initial step, iodine attacks the carbonyl oxygen of aldehyde and gives rise to the reaction, thereby carbonyl carbon gets bonded with nitrogen of γ-butyrolactam to form an *N*-acyliminium cation intermediate. Nucleophilic attack of thiophenol on the *N*-acyliminium cation yields the desired product (**4**). A possible mechanism for this reaction is proposed in Scheme 1.

For the synthesis of product (**4**), two mechanisms are possible. One possible mechanism is the formation of thiohemiacetal in the first step and the second step is the attack of amide on thiohemiacetal to yield the desired product. Another possible mechanism is the generation of an *N*-acyliminium ion in the first step followed by the attack of nucleophile (thiophenol) on the *N*-acyliminium ion leading to the formation of the desired product. In order to elucidate the exact mechanism, we carried out two reactions. One was the reaction between γ-butyrolactam and benzaldehyde, and the other was between thiophenol and benzaldehyde under the same conditions with 15 mol% iodine in DCM. In the former reaction we added thiophenol to the reaction mixture after 30–40 min and got the product (**4**), if the reaction was allowed to proceed without adding thiophenol it gives 1,1′-((2-bromophenyl)methylene)bis(pyrrolidin-2-one) product (**5**).[10a] This reaction scheme and its mechanism are represented in Schemes 2 and 3 respectively.

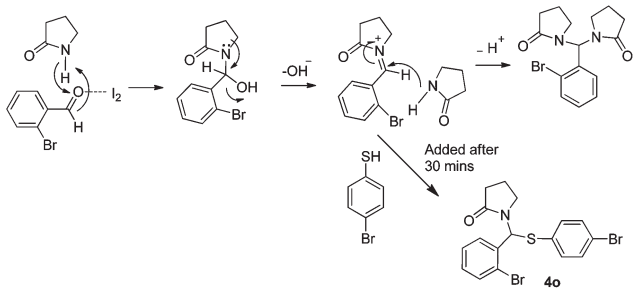
In the latter one we added γ-butyrolactam to the reaction mixture after 30–40 min but did not get the product (**4**). Instead of getting product (**4**) we got (phenylmethylene)bis((4-methoxy phenyl)sulfane) product (**6**).[10b] The reaction scheme and its mechanism are represented in Schemes 4 and 5 respectively.

Hence we have concluded that the mechanism for this reaction would proceed through only an *N*-acyliminium intermediate. Finally we corroborated the mechanism by recording LC-Ms for the *N*-acyliminium intermediate formed during the reaction between γ-butyrolactam and 2-fluorobenzaldehyde which strongly confirms the presence of an *N*-acyliminium intermediate as shown in Fig. 1.

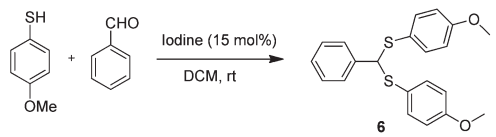
We found out the optimal conditions for the three components (**1–3**) domino reaction and carried out these reactions with a diverse range of aromatic aldehydes and thiophenols to explore the generality of the reaction in other systems as shown in Table 3.

The structure of compound **4j** was further confirmed by the single crystal X-ray diffraction analysis as shown in Fig. 2.

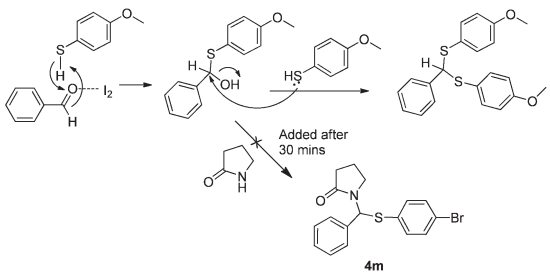
We also investigated the scope and the limitation of this domino reaction. We found that aldehydes possessing electron-withdrawing groups (EWG) on the benzene ring gave the product in excellent yield within a short reaction time, whereas electron donating groups (EDG) in aldehydes gave a lower yield and took a longer time. This is because EWG in aromatic aldehydes allow the aldehyde group to react more rapidly with



Scheme 3 A possible mechanism for the formation of product (**4** and **5**).



Scheme 4 Reaction of γ-butyrolactam and benzaldehyde.



Scheme 5 A possible mechanism for the formation of product (**6**).

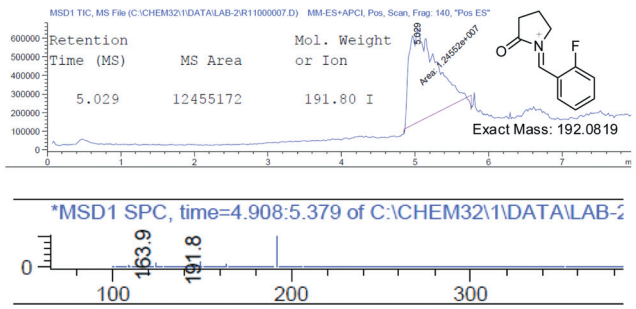


Fig. 1 LC-Ms spectra of an *N*-acyliminium intermediate from compound **4g**.

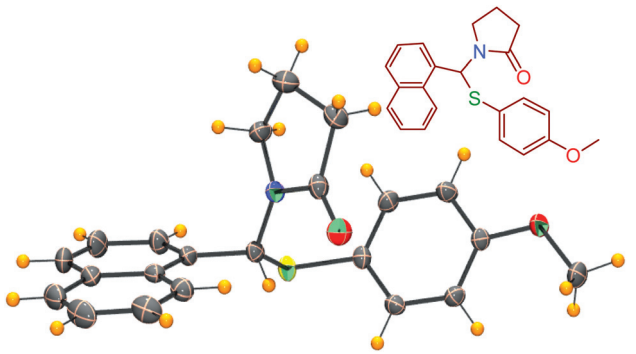


Fig. 2 The ORTEP diagram of compound **4j** as obtained by X-ray crystallography.

Table 3 *In vitro* anticancer studies for the synthesised analogue against different cancer cell lines

		Concentration (IC_{50} in μM)[a]						
Entry	Code	ACHN Renal cancer	PANC1 Pancreatic cancer	CALU-1 Lung cancer	H460 Non-small cell lung cancer	HCT116 Colon cancer	MCF7 Breast cancer	MCF10A Normal breast epithelium
1	**4b**	1.6 ± 0.13	1.5 ± 0.32	2.6 ± 0.24	2.7 ± 0.27	1.8 ± 0.16	2.3 ± 0.27	9.8 ± 0.63
2	**4f**	0.9 ± 0.07	0.8 ± 0.06	0.9 ± 0.08	1.1 ± 0.13	1.3 ± 0.12	2.4 ± 0.19	9.6 ± 0.57
3	**4g**	1.2 ± 0.21	0.9 ± 0.1	1.1 ± 0.12	1.06 ± 0.14	0.9 ± 0.08	1.3 ± 0.09	11.34 ± 0.94
4	**4i**	0.7 ± 0.06	1.2 ± 0.17	0.6 ± 0.05	0.8 ± 0.05	0.9 ± 0.08	1.2 ± 0.12	14.87 ± 1.52
5	**4j**	3.1 ± 0.39	2.9 ± 0.65	3.3 ± 0.22	3.5 ± 0.44	2.2 ± 0.24	3.6 ± 0.38	14.7 ± 0.89
6	**4m**	2.5 ± 0.42	1.9 ± 0.14	2.1 ± 0.17	2.3 ± 0.15	1.7 ± 0.36	2.6 ± 0.17	10.4 ± 0.78
7	**4n**	2.1 ± 0.32	1.4 ± 0.16	1.5 ± 0.21	2.4 ± 0.33	2.7 ± 0.42	3.3 ± 0.25	15.6 ± 0.92
8	**4o**	0.6 ± 0.05	0.64 ± 0.07	0.8 ± 0.07	1.1 ± 0.12	1.2 ± 0.14	1.7 ± 0.14	8.9 ± 0.73

[a] The experiment was performed in triplicate for three repeats, and IC_{50} values were expressed as mean ± SEM.

the nitrogen atom of γ-butyrolactam to form an *N*-acyliminium cation (intermediate) in a more stable system leading to a faster reaction and giving a higher yield, while in aromatic aldehydes containing EDG the formation of an *N*-acyliminium cation is less stable leading to a slower reaction and lower yield.

We have evaluated *in vitro* anticancer activity for the synthesized molecule at four different concentrations of 0.3 μM, 1 μM, 3 μM and 10 μM against a panel of five cancer cell lines. The human tumor cell lines of renal cancer, pancreatic cancer, lung cancer, colon cancer and normal breast epithelium of MCF7 and MCF10A were used for evaluating anticancer activity on the high throughput screening platform using Cell Counting Kit (CCK8) cell proliferation and cytotoxicity assays.

A preliminary screening showed that some of the derivatives exhibited moderate to strong anticancer activity on various cancer cell lines which have been shown in the ESI.†

For those drug molecules that are active against cancer cells in the preliminary screening, we studied their molecular stability in stimulated gastric fluid[11] and bovine serum albumin.[12] SGF was prepared according to the US Pharmacopeia (USP XII 1995).[11a] BSA with high purity (>98%) was purchased from SRL Pvt. Ltd which was made fatty acid free by using Norit[12a] and prepared following the standard method.[12b]

The molecular stability was measured by UV-Visible spectra (λ = 400–190 nm) for an interval of time in SGF and BSA as shown in the ESI.† At different drug concentrations a linear calibration curve was plotted under similar conditions from which the sample concentrations were calculated.

The molecular stability was measured by the percentage of concentration loss in comparison to the freshly prepared known samples. Some of the drug molecules showed moderate to strong stability towards SGF and BSA for 4–5 h. Detailed stability studies of SGF and BSA for compounds **4b**, **4f**, **4g**, **4i**, **4j** and **4m–o** using UV-Visible spectra have been given in the ESI.†

We evaluated IC_{50} values of primary active compounds at 8 different concentrations (0.003 μM, 0.01 μM, 0.03 μM, 0.3 μM, 1 μM, 3 μM, 10 μM and 30 μM) to study the activity against cancer cell lines at varied concentrations. The results revealed that the compound **4i** showed potent IC_{50} in the range of 0.6 μM to 1.2 μM in cancer cells, while IC_{50} of normal breast epithelium cells showed 14.87 μM for MCF10A and 1.2 μM for MCF7. Hence, **4i** was highly active towards proliferating cells and even other compounds **4f**, **4o** and **4g** were found to be potentially active against cancer cell lines with less cytotoxicity on breast cancer cells as well as normal epithelium cells. The results obtained for the active analogues are reported in Table 3. In most of the cases these analogues were found to possess good activity against cancer cells, if aromatic aldehydes contain EWG.

In summary, we have successfully developed an efficient synthetic protocol for a domino reaction of γ-butyrolactam, aromatic aldehydes and substituted thiophenols using iodine as a catalyst. The Lewis acidity of iodine shows enormous catalytic activity making it capable of binding with the carbonyl oxygen of aldehyde to yield the desired product. These synthesized pyrrolidone (**4**) analogues have been evaluated for their anticancer activity and their stability in SGF and BSA. A few of these compounds were found to be effective against different cancer cell lines. Thus we conclude that these reactions offer several advantages such as readily available starting materials, flexible substitution patterns, mild reaction conditions and a convenient work-up.

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