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Cytostatic activity of novel 4′-aminochalcone-based imides

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Abstract—A series of nine 1-(4-((*E*)-3-arylacryloyl)phenyl)-1*H*-pyrrole-2,5-diones **3a–i** (4′-aminochalcone-based maleimides) was synthesized as candidate cytotoxic agents. The efficacy of these potential cytotoxics were evaluated against three representative cell lines and more than half of the drug candidates proved to exhibit significant cytostatic activity in vitro. QSAR studies using statistical analyses on several physicochemical parameters and IC_{50} values resulted in a few very important correlations which will aid in later the amplification of the project. Representative test compounds were well tolerated by mice in in vivo survival and toxicity studies.

Chemists have long sought a 'magic bullet' for the treatment of cancer, a compound that will selectively kill cancerous cells without affecting the normal cells. A number of chalcones (i.e., 1,3-diaryl-2-propen-1-ones) have demonstrated cytotoxic and anticancer properties because of their preferential reactivity toward cellular thiols in contrast to amino and hydroxy groups found in nucleic acids.[1–4] Hence, these compounds may be free from the problems of mutagenicity and carcinogenicity that are associated with a number of alkylating agents used in cancer chemotherapy.

Thiol-alkylators such as chalcones are most commonly associated with enzymes; in particular, topoisomerase II which is unusually rich in cysteine residues.[5] Topoisomerases participate in DNA replication by facilitating cleavage of the DNA template. Any interruption in this intricate process can lead to potentially lethal DNA strand breaks, which may induce apoptosis.[6] The affinity of thiol-alkylators for topoisomerase II, a regulator of DNA topology, offers a means of selectivity.[7,8] In the case of thiol-alkylators, such as α,β-unsaturated ketones, inhibition of tumor growth arises through nucleophilic addition involving reactive thiols of key regulatory enzymes.

Pharmacological activity of cyclic imides, a type of cyclic α,β-unsaturated ketones, has been extensively explored,[9–14] such as the F-ring of the indolocarbazole antitumor drug NB-506 (Fig. 1) which was found to increase the cytotoxicity, DNA binding, and topoisomerase I inhibition activities.[14] *N*-Methyl maleimide (NMM, Fig. 1) and *N*-ethyl maleimide (NEM, Fig. 1) are also examples of potent thiol-alkylators of topoisomerase II and have been widely investigated.[5,15,16]

The present work was aimed at investigating the cytotoxic efficacy of novel electrophilic thiol-alkylators related to 4′-aminochalcone (**1**). We have reported the cytotoxic potencies of 4′-aminochalcones (**1a–d**, **g**; Fig. 1) and 4′-aminochalcone maleamic acids (**2a–g**; Fig. 1) against human Molt 4/C8 and CEM T-lymphocytes as well as murine P388 and L1210 leukemic cells.[17] The molecules **3a–i** (Fig. 1) are derived from the hybridization of two kinds of α,β-unsaturated ketones (Michael acceptors), a chalcone and a maleimide, both of which have shown notable anti-cancer properties independently.[5,15–20] Our design seeks to optimize the potency of single Michael acceptors by providing multiple sites of reactivity toward cellular thiols. Importantly, these mild alkylating agents address the issue of

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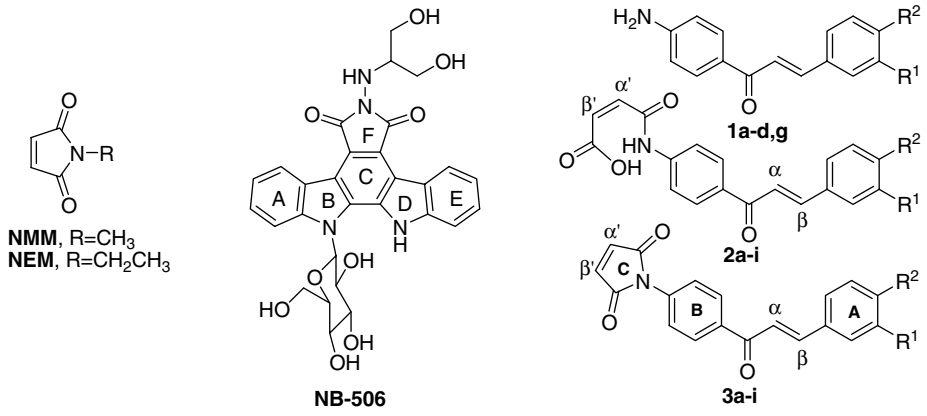


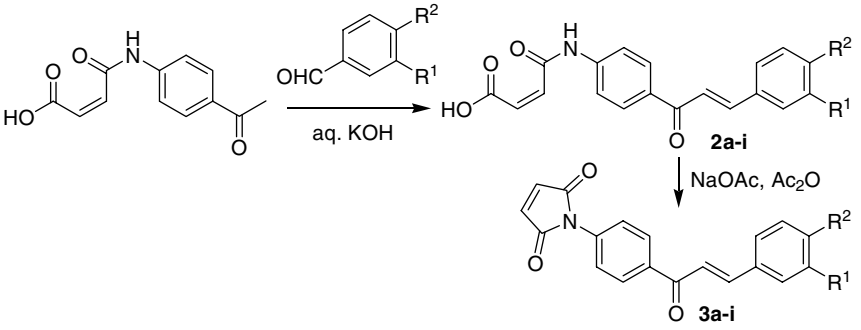
Figure 1. Molecular structures of NMM, NEM, NB-506, and 4′-aminochalcone derivatives **1**–**3**. The aryl substituents in series **1**–**3** are as follows: **a**: $R^1 = R^2 = H$; **b**: $R^1 = H$, $R^2 = Cl$; **c**: $R^1 = R^2 = Cl$; **d**: $R^1 = H$, $R^2 = CH_3$; **e**: $R^1 = H$, $R^2 = OCH_3$; **f**: $R^1 = R^2 = OCH_3$; **g**: $R^1 = H$, $R^2 = NO_2$; **h**: $R^1 = H$, $R^2 = CO_2H$; **i**: $R^1 = R^2 = –OCH_2O–$.

genotoxicity that is characteristic of non-specific cellular alkylators.

Series **3a**–**i** are second generation derivatives aimed at enhancing the cytotoxic potency of compounds in series **2**. Drawbacks of the compounds in series **2** include ionization of the maleamic carboxylic acid which slows their transmembrane movement.[17] In series **3**, the maleamic carboxylate of series **2** was replaced by the maleimide functionality. The retained chalcone moiety allows tuning of electronic and steric properties as well as lipophilic character. Although both series of compounds (**2** and **3**) have two electrophilic alkene sites (β-carbon atom and either the α′ or β′-carbons, Fig. 1) susceptible to Michael additions involving cellular thiols, compounds of series **3** are expected to show enhanced electrophilicity owing to maleimide moiety. Maleimide ring is anti-aromatic (4π electrons) and strained (bond angles ~108° as opposed to 120° for sp^2 C), and is therefore less stable. Thiolation of maleimide ring will not only release some angular strain, but it will also result into an aromatic system when viewed in enolic form. The two electrophilic α,β-unsaturated ketones will likely allow sequential alkylation of two reactive thiols by a single agent. Sequential chemical insults result in enhanced cytotoxicity against tumor cells relative to healthy cells.[21] Hence the compounds in series **3** should have the potential to display preferential cytotoxicity for malignant cells. In particular, if cytotoxicity was influenced by the chemical reactivity of the enone group, a correlation between the IC_{50} values of **3a**–**i** and Hammett σ values and/or partial atomic charges on electrophilic carbons should emerge.

The target compounds were prepared as shown in Scheme 1. Claisen-Schmidt condensation of 4′-acetyl-*N*-maleanilinic acid and various aryl aldehydes under basic conditions afforded the 4′-aminochalcone-maleamic acids **2a**–**i**.[17] The reaction between 4′-aminoacetophenone and maleic anhydride produced the precursor 4′-acetyl-*N*-maleanilinic acid.[22] The desired chalcone-imides **3a**–**i** were obtained on dehydration of 4′-aminochalcone-maleamic acids **2a**–**i**. New compounds viz. **2h**, **2i**, and **3a**–**i** were completely characterized based on their spectroscopic data.[23]

All the compounds in chalcone-imide series **3** were evaluated against human Molt 4/C8 and CEM T-lymphocytes as well as against L1210 leukemia cells following a literature procedure.[24] These data are presented in Table 1. The clinically approved cytotoxic drug Alkeran® (i.e., melphalan) was used as a reference drug which is also known to sequentially conjugate with sulfhydryl groups.[25] Three representative compounds (**3b**, **3e**, and **3h**) were also evaluated in short-term survival and neurotoxicity assays in mice.



Scheme 1. Synthetic scheme for preparing compounds **2a**–**i** and **3a**–**i**. R^1 and R^2 substituents are indicated in Figure 1 legend.

Table 1. Cytostatic potency of compounds **3a–i** against human Molt 4/C8 and CEM T-lymphocytes and murine L1210 cell lines

Compound	IC_{50} (μM)		
	L1210	Molt 4/C8	CEM
3a	8.6 ± 2.2	9.5 ± 0.1	9.8 ± 0.3
3b	7.3 ± 0.4	7.5 ± 1.0	7.5 ± 0.3
3c	6.5 ± 0.9	8.8 ± 0.5	7.7 ± 0.7
3d	1.5 ± 0.7	2.4 ± 0.6	4.1 ± 2.7
3e	9.8 ± 6.7	7.9 ± 0.5	8.4 ± 0.3
3f	157 ± 0.7	202 ± 36	220 ± 1
3g	>500	>500	>500
3h	135 ± 99	178 ± 6	133 ± 97
3i	160 ± 99	214 ± 47	258 ± 69
Alkeran®[a]	2.13 ± 0.03	3.24 ± 0.79	2.47 ± 0.03

[a] The data for Alkeran® are reproduced from the *Eur. J. Med. Chem.* 35, 970 (2000).

Human Molt 4/C8 and CEM T-lymphocyte cell lines were chosen to obtain an appreciation of the toxicity of these compounds toward human mutated cells. The murine L1210 cytotoxicity assay is regarded as a predictor of clinically useful anticancer drugs.[26] From the data in Table 1, the following observations were made: first, nearly half of the compounds (**3a–e**) displayed noteworthy cytostatic activity against the three cell lines. Although slightly inferior to Alkeran®, compounds **3a–c** and **3e** showed cytotoxic potencies of the same order of magnitude. Second, compound **3d** was as potent as Alkeran® against all three cell lines when standard deviations were taken into consideration. There was a strong correlation of the cytostatic potency between the three tumor cell lines. This observation points to a common molecular mechanism of cytostatic activity that is present across three different tumor cell lines. Compounds **3f–i** were essentially inactive. With comparable IC_{50} values for the cytostatic potential of compounds **3a–c** and their precursors **1a–c**,[17] the presence of maleimido group on **3a–c** did not appear to significantly influence the bioactivity. However, the tolyl analog **3d** was found to be 6.9, 3.5, and 2.1 times more active than the corresponding 4′-aminochalcone analog **1d** for L1210, Molt 4/C8, and CEM lymphocytic cell lines, respectively.

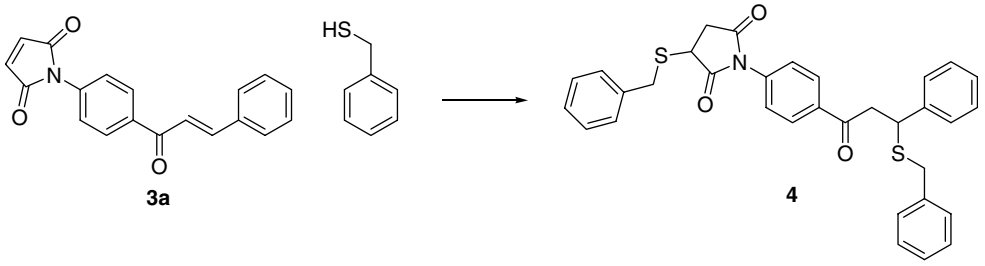
To obtain experimental support for the assertion that these compounds act via thiolation of sulfhydryl-rich proteins such as topoisomerase 2α, a representative example, **3a**, was reacted with two equivalents of benzyl mercaptan under aqueous buffered condition at physiological pH and temperature. This resulted in formation of di-benzylthio adduct (**4**, Scheme 2)[23c] which qualitatively substantiates our claim of sequential chemical insult by these types of compounds to cellular thiols.

The disparity in activity in highly related compounds (Table 1) required a systematic quantitative structure-activity relationship (QSAR)[27] study to understand the effect of structural and electronic properties of the molecule on cytostatic potency. Physicochemical parameters of the molecule and/or aromatic substituents such as calculated partial charge,[28] calculated partition coefficients ($c\log P$),[28] Hammett σ,[29] Hansch π[29], and molar refractivity (MR)[29] were used for statistical correlation studies. The calculated atomic charges at various electrophilic carbon atoms of compounds **3a–i** are presented in Table 2. The partial atomic charges on carbons designated α′ and β′ (Fig. 1 and Table 2) were found to be nearly identical across the series presumably because substitutions on aromatic ring **A** have little or no electronic effect on these carbons, therefore these values were not used for QSAR studies.

Linear and semilogarithmic correlations were generated[30] for the IC_{50} of each cell line and physicochemical parameters using statistical analysis. The only significant correlations obtained are tabulated in Table 3. The value of P in the range 0.1–0.05 shows a trend toward significance, while values <0.05 are indicative of significant correlation. The magnitude of coefficient r indicates the extent of correlation (the closer the values to 1, the better), while the sign indicates whether the correlation is positive or negative. It was logical to assume that adding electron-withdrawing groups appropriately placed would increase the potency of **3a–i** since electron-with-

Table 2. Atomic charges of chalcone-imides

Compound	Partial atomic charges (esu)			
	α	β	α′	β′
3a	−0.252	0.007	−0.133	−0.136
3b	−0.246	0.003	−0.134	−0.134
3c	−0.241	−0.002	−0.133	−0.135
3d	−0.256	0.014	−0.135	−0.137
3e	−0.262	0.021	−0.134	−0.137
3f	−0.248	0.005	−0.135	−0.135
3g	−0.214	−0.027	−0.132	−0.134
3h	−0.248	0.005	−0.133	−0.134
3i	−0.250	0.005	−0.134	−0.135



Scheme 2. Formation of thiol adduct **4**. Reagents and condition: pH 7.4 MOPS buffer, DMSO, 37 °C.

Table 3. Pearson correlation between IC_{50} values of chalcone-based imides **3a–i** and the physicochemical parameters

Independent	Dependent	Type	*r* Coefficient	*P* value
Charge β	Molt4 IC_{50}	Linear	−0.795	0.010
	CEM IC_{50}	Linear	−0.776	0.014
	L1210 IC_{50}	Linear	−0.836	0.005
c Log *P*	Molt4 IC_{50}	Semi-log	−0.657	0.055
	CEM IC_{50}	Semi-log	−0.677	0.045
	L1210 IC_{50}	Semi-log	−0.681	0.044
Hansch π	Molt4 IC_{50}	Semi-log	−0.674	0.046
	CEM IC_{50}	Semi-log	−0.685	0.042
	L1210 IC_{50}	Semi-log	−0.700	0.036

drawing groups increase the electrophilicity of the conjugated unsaturated ketones and, therefore, promote the attack by biological nucleophiles. A negative correlation of IC_{50} with partial charge on β-carbon atom (Table 3) indicates a decrease in IC_{50} of chalcone-imides with rise in electrophilicity of the β-carbon atom. Based on values in Table 2, it can be noted that in case of **3a–i**, attack by cellular nucleophiles will take place formerly at the β-carbon atom followed by α′ or β′-carbon atoms of the imide ring. This explains the possibility of compounds in series **3** undergoing a cascade of chemical alkylations using cellular nucleophilic thiols.

Negative correlation of both *c* Log *P* and Hansch π with cytostatic activity infers that when lipophilicity increases, the IC_{50} decreases leading to improved cytotoxicity. This is clearly evident from the lack of cytostatic activity in dimethoxy, nitro, carboxy, and methylenedioxy substituted polar compounds (**3f–i**, respectively) presumably because of their difficulty in crossing the lipid membrane of cancerous cells. Therefore, adding more hydrophobic groups would be a good start for further analog preparation.

It is recognized that a number of tumors are slightly more acidic than the corresponding healthy cells due to increased anaerobic glycolysis in malignant cells.[31] Therefore, compounds **3a–i** can potentially hydrolyze (chemically/enzymatically) to their chalcone maleamic acid (**2**) counterparts. The contention, whether the observed activity was due to the parent compounds per se or due to their possible hydrolyzed products, was considered. We have recently reported the cytotoxicity of these chalcone maleamic acids (**2a–g**)[17] which are the synthetic precursors to compounds **3a–g** (Scheme 1). When compared to the reported cytotoxic potential of precursor **2a–e**, cyclization to imide clearly results in significantly increased potency in compounds **3a–e**; the observed cytotoxicity, therefore, is not due to the hydrolyzed products.

Three representative compounds **3b**, **3e**, and **3h** were also examined for murine toxicity in vivo following literature procedure.[32] These compounds were injected intraperitoneally into mice at doses of 30, 100, and 300 mg/kg. After the administration of the compounds, the animals were observed for 0.5 and 4 h. Mortality was observed only in case of compound **3h** at a dose of 300 mg/kg and after 4 h of sample injection. Neurotoxicity was measured using the rotating rod procedure[33]; in addition, mice were observed for other pathological symptoms. Neurological deficit displayed after 0.5 h (in parentheses are the number of animals displaying neurotoxicity/total number of animals, dose of compound) namely **3b** (1/8, 100; 2/4, 300) and **3h** (1/8, 100; 2/4, 300). The neurotoxicity displayed after 4 h by the compound, namely **3h**, is (1/4, 100; 2/2, 300). The mice which received the dose of 300 mg/kg of **3h** for 4 h succumbed to death. No neurotoxicity was observed for **3e**, suggesting that the 4-methoxy analog **3e** was well tolerated, while the presence of the 4-COOH analog **3h** appeared to be detrimental.

In conclusion, the present investigation revealed 4′-aminochalcone-based maleimides as potential leads for development of novel cytotoxics. Compound **3d** proved at least as potent as the reference drug Alkeran® against L1210, Molt C4/8, and CEM cells. QSAR results also provide useful suggestions for modifying the parent model. The in vivo toxicity experiments reveal that the chalcone imides are not general biocidal agents. Substantial structural modifications of chalcone-imides have important potential for further drug development as anti-cancer agents.

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23. (a) Analytical data for **2h**: yield 60%, mp 195–200 °C, ^{1}H NMR (DMSO-d_6): δ 6.38 (1H, d, J = 12.0 Hz, *Z*-vinylic-H), 6.60 (1H, d, J = 12.0 Hz, *Z*-vinylic-H), 7.76 (1H, d, J = 15.6 Hz, *E*-vinylic-H), 7.84 (2H, d, J = 8.5 Hz, Ar-H), 7.91–8.03 (4H, m, Ar-H), 8.07 (1H, d, J = 15.6 Hz, *E*-vinylic-H), 8.19 (2H, d, J = 8.5 Hz, Ar-H), 11.00 (1H, s, NHCO); ^{13}C NMR (DMSO-d_6): δ 123.20, 128.53, 133.22, 134.06, 134.36, 135.25, 136.34, 136.76, 143.24, 146.34, 147.77, 168.03, 171.19, 171.45, 191.84; IR (KBr): ν 3447, 1734, 1718, 1700, 1696, 1603, 1560, 1384, 1326 cm^{-1}; UV–vis (MeOH): λ_{max} 310 nm; ESI-MS $(M-H)^-$ Calcd: 365.09, found: 363.90. For **2i**: yield 79%; mp 198–200 °C; ^{1}H NMR (DMSO-d_6): δ 6.12 (2H, s, OCH_2O), 6.36 (1H, d, J = 12.0 Hz, *Z*-vinylic-H), 6.54 (1H, d, J = 12.0 Hz, *Z*-vinylic-H), 7.00 (1H, d, J = 8.1 Hz, Ar-H), 7.15–7.60 (2H, m, Ar-H), 7.64–7.95 (5H, m, *E*-vinylic-H, Ar-H), 8.17 (1H, d, J = 8.6 Hz, Ar-H), 10.74 (1H, s, NHCO); ^{13}C NMR (DMSO-d_6): δ 102.51, 107.78, 109.39, 119.62, 120.73, 126.71, 130.15, 130.68, 131.21, 132.36, 133.73, 143.83, 144.38, 148.96, 150.35, 164.56, 167.86, 188.26; IR (KBr): ν 3447, 3286, 3208, 3118, 1709, 1647, 1631, 1593, 1534, 1500, 1384, 1360, 1262, 1243, 1177, 1033, 847, 834, 802 cm^{-1}; UV–vis (MeOH): λ_{max} 362 nm; ESI-MS $(M-H)^-$ Calcd: 365.09, found: 363.90; (b) General procedure for synthesis of compounds **3a**–**i**. A mixture of anhydrous NaOAc (5 mmol) and acetic anhydride (20 mmol) was heated at 90 °C with stirring until all NaOAc had dissolved. The appropriate 4′-aminochalcone maleamic acid analog **2a**–**i** was added and stirred at the noted temperature range for 15 min. After completion of reaction the mixture was allowed to cool to room temperature and the reaction mixture quenched with ice cold water. The precipitate was filtered and dried, and the crude product was purified by column chromatography using DCM as eluent. Analytical data for **3a:** yield 17%; mp 197–200 °C; ^{1}H NMR (DMSO-d_6): δ 7.25 (2H, s, pyrrole-Hs), 7.47–7.49 (3H, m, Ar-H), 7.58 (2H, d, J = 8.5 Hz, Ar-H), 7.78 (1H, d, J = 15.7 Hz, *E*-vinylic-H), 7.90–7.93 (2H, m, Ar-H), 7.98(1H, d, J = 15.7 Hz, *E*-vinylic-H), 8.28 (2H, d, J = 8.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6): δ 122.83, 127.17, 129.82, 130.07, 131.62, 135.47, 135.77, 136.58, 137.06, 145.17, 170.43, 189.32; IR (KBr): ν 3435, 3083, 1704, 1662, 1604, 1384, 1307 cm^{-1}; UV–vis (MeOH): λ_{max} 220, 318 nm; HRMS Calcd: 303.0895, found: 303.0899. For **3b**: yield 56%; mp 175–177 °C; ^{1}H NMR (DMSO-d_6): δ 6.93 (2H, s, pyrrole-Hs), 7.42 (2H, d, J = 8.2 Hz, Ar-H), 7.51(1H, d, J = 15.7 Hz, *E*-vinylic-H), 7.58–7.63 (4H, m, Ar-H), 7.80 (1H, d, J = 15.9 Hz, *E*-vinylic-H), 8.13 (2H, d, J = 8.4 Hz, Ar-H); ^{13}C NMR (DMSO-d_6): δ 119.64, 123.56, 127.15, 129.84, 130.11, 131.39, 131.52, 135.78, 136.94, 143.65, 170.42, 189.18; IR (KBr): ν 3427, 3071, 1716, 1660, 1608, 1384, 1305 cm^{-1}; UV–vis (MeOH): λ_{max} 204, 226, 324 nm; HRMS Calcd: 337.0505, found: 337.0501. For **3c**: yield 94%; 207–210 °C; ^{1}H NMR (DMSO-d_6): δ 7.25 (2H, s, pyrrole-Hs), 7.59 (2H, d, J = 8.5 Hz, Ar-H), 7.71–7.77 (2H, m, Ar-H, *E*-vinylic-H), 7.89–7.92 (1H, m, Ar-H), 8.09 (1H, d, J = 15.6 Hz, *E*-vinylic-H), 8.29–8.31 (3H, m, Ar-H); ^{13}C NMR (DMSO-d_6): δ 124.80, 127.15, 130.12, 130.22, 131.09, 131.90, 132.70, 133.68, 135.80, 136.41, 136.74, 136.79, 142.30, 170.43, 189.02; IR (KBr): ν 3427, 3071, 1720, 1637, 1608, 1384, 1305 cm^{-1}; UV–vis (MeOH): λ_{max} 204, 207 nm; HRMS Calcd: 371.0116, found: 371.0093. For **3d**: yield 50%; mp 178–181 °C; ^{1}H NMR (DMSO-d_6): δ 3.37 (3H, s, CH_3), 7.24 (2H, s, pyrrole-Hs), 7.28 (2H, d, J = 8.1 Hz, Ar-H), 7.56 (2H, d,J = 8.5 Hz, Ar-H), 7.75 (1H, d, J = 15.8 Hz, *E*-vinylic-H), 7.80 (2H, d, J = 8.1 Hz, Ar-H), 7.80 (1H, d, J = 15.6 Hz, *E*-vinylic-H), 8.26 (2H, d, J = 8.5 Hz, Ar-H); ^{13}C NMR (DMSO-d_6): δ 21.97, 121.75, 127.11, 129.86, 130.00, 130.43, 132.78, 135.75, 136.49, 137.18, 141.71, 145.22, 170.43, 189.22; IR (KBr): ν 3427, 3071, 1716, 1660, 1608, 1384, 1305 cm^{-1}; UV–vis (MeOH): λ_{max} 231, 332 nm; HRMS Calcd: 317.1052, found: 317.1050. For **3e**: yield 99%; 165–167 °C; ^{1}H NMR (DMSO-d_6): δ 3.88 (3H, s, OCH_3), 6.97 (2H, d, J = 8.6 Hz, Ar-H), 7.28 (2H, s, pyrrole-Hs), 7.41 (1H, d, J = 15.7 Hz, *E*-vinylic-H), 7.56–7.65 (4H, m, Ar-H), 7.82 (1H, d, J = 15.7 Hz, *E*-vinylic-H), 8.13 (2H, d, J = 8.4 Hz, Ar-H); ^{13}C NMR (DMSO-d_6): δ 56.27, 115.31, 120.29, 127.13, 129.93, 131.76, 135.76, 136.37, 137.37, 145.17, 170.45, 189.11; IR (KBr): ν 3431, 3093, 1712, 1660, 1608, 1384, 1304, 1030 cm^{-1}; UV–vis (MeOH): λ_{max} 204, 235, 347 nm; HRMS Calcd: 333.1001, found: 333.0989. For **3f**: 92%; mp 200–201 °C; ^{1}H NMR (DMSO-d_6): δ 3.83 (6H, s, OCH_3), 7.25 (2H, s, pyrrole-Hs), 7.41 (2H, d, J = 8.4 Hz, Ar-H), 7.86 (1H, d, J = 15.6 Hz, *E*-vinylic-H), 7.58–7.63 (4H, m, Ar-H), 8.26 (1H, d, J = 15.3 Hz, *E*-vinylic-H), 7.58 (2H, d, J = 8.4 Hz, Ar-H); ^{13}C NMR (DMSO-d_6): δ 56.48, 56.61, 111.65, 112.43, 120.37, 124.97, 127.11, 128.30, 129.94, 135.77, 136.37, 137.41, 145.69, 149.89, 152.26, 170.45, 189.14; IR (KBr): ν 3447, 2926, 1718, 1654, 1605, 1384, 1015 cm^{-1}; UV–vis (MeOH): λ_{max} 204, 235, 347 nm; HRMS Calcd: 363.1106, found: 363.1117. For **3g**: 40%; mp 200–202 °C; ^{1}H NMR (DMSO-d_6): δ 7.25 (2H, s, pyrrole-Hs), 7.61 (2H, d, J = 8.5 Hz, Ar-H), 7.86 (1H, d, J = 15.7 Hz, *E*-vinylic-H), 8.15–8.21 (3H, m, *E*-vinylic-H and Ar-H), 8.30–8.33 (4H, d, J = 8.6 Hz, Ar-H); ^{13}C NMR (DMSO-d_6): δ 124.83, 126.85, 127.18, 130.28, 130.81, 135.81,

136.62, 136.91, 142.00, 142.22, 149.00, 170.41, 189.14; IR (KBr): ν 3427, 3071, 1716, 1660, 1608, 1384, 1305 cm^{-1}; UV–vis λ_{max} (MeOH): 219, 222, 328 nm; HRMS Calcd: 348.0746, found: 348.0745. For **3h**: yield 82%; 270–273 °C; ^1H NMR(DMSO-d_6): δ 7.25 (2H, s, pyrrole-Hs), 7.59 (2H, d, J = 8.2 Hz, Ar-H), 7.81 (1H, d, J = 15.6 Hz, *E*-vinylic-H), 8.09 (1H, d, J = 15.7 Hz, *E*-vinylic-H), 8.02 (4H, m, *E*-vinylic-H and Ar-H), 8.30 (2H, d, J = 8.4 Hz, Ar-H); ^{13}C NMR (DMSO-d_6): δ 124.98, 127.17, 129.84, 130.18, 130.59, 133.07, 135.79, 136.76, 136.82, 139.98, 143.65, 167.72, 170.42, 189.22; IR (KBr): ν 3427, 3080, 1715, 1664, 1609, 1384, 1292 cm^{-1}; UV–vis (MeOH): λ_{max} 205, 322 nm; HRMS Calcd: 347.0793, found: 347.0790. For **3i**: yield 34%; mp 197–198 °C; ^1H NMR (DMSO-d_6): δ 6.06 (2H, s, OCH_2O), 6.88–7.20 (3H, m, Ar-H), 7.28 (2H, s, pyrrole-Hs), 7.38 (1H, d, J = 15.6 Hz, *E*-vinylic-H), 7.58 (2H, d, J = 8.4 Hz, Ar-H), 7.78 (1H, d, J = 15.3 Hz, *E*-vinylic-H), 8.13 (2H, d, J = 8.4 Hz, Ar-H); ^{13}C NMR (DMSO-d_6): δ 102.56, 107.85, 109.43, 120.73, 127.00, 127.12, 129.97, 130.03, 135.77, 136.43, 137.29, 145.19, 148.99, 150.56, 170.44, 189.01; IR (KBr): ν 3447, 3071, 1714, 1650, 1608, 1384, 1305 cm^{-1}; UV–vis (MeOH): λ_{max} 269 nm; HRMS Calcd: 347.0793, found: 347.0778; (c) Synthesis of 1-(4-(3-(benzylthio)-3-phenylpropanoyl)phenyl)-3-(benzylthio)pyrrolidine-2,5-dione (**4**). To a suspension of **3a** (0.59 mmol) in MOPS (3-(*N*-morpholino)propanesulfonic acid) buffer (100 mM, 10 ml, pH 7.4) and DMSO (1 mL), benzyl mercaptan (1.20 mmol) was added and the resulting mixture stirred at 37 °C for 72 h. Reaction mixture was worked up by adding water (75 ml) and extracting with ethyl acetate (3× 25 ml). Organic layer was dried over anhyd Na_2SO_4 and concentrated to give crude product which was purified by column chromatography (13% EtOAc–hexane eluent) as colorless oil in 50% yield. Analytical data for **4:** ^1H NMR δ ($CDCl_3$): 2.57 and 2.64 (1H, 2d, J = 3.6 Hz each, $CH_\alpha H_\beta$), 3.15 and 3.22 (1H, dd, J = 9.3 and 18.9 Hz, $CH_\alpha H_\beta$), 3.50 (2H, m, CH_2), 3.55 (2H, d, J = 8.4 Hz, CH_2), 3.64 and 3.68 (1H, 2d, J = 3.6 Hz each, CH), 3.92 and 4.29 (1H each, d, J = 13.8 Hz each, $CH'_\alpha H'_\beta$), 7.24–7.46 (17H, m, ArH), 7.97 (2H, d, J = 8.4 Hz, ArH); ^{13}C NMR δ ($CDCl_3$): 30.12, 35.89, 36.50, 37.66, 44.51, 45.74, 126.71, 127.46, 127.83, 128.09, 128.43, 128.89, 129.00, 129.19, 129.33 (2C), 129.66, 136.10, 136.80, 136.95, 138.23, 141.92, 173.54, 175.58, 196.11; IR (KBr): ν 3054, 1720, 1691, 1603, 1494, 1421, 1377, 1265, 1178, 895 cm^{-1}; UV–vis (MeOH): λ_{max} 254 nm; HRMS calculated for $C_{33}H_{29}NO_3S_2Na^+$ 574.1481, found: 574.1450.

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