Bioorganic & Medicinal Chemistry 22 (2014) 1672–1679

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of 2,5-di(7-indolyl)-1,3,4-oxadiazoles, and 2- and 7-indolyl 2-(1,3,4-thiadiazolyl)ketones as antimicrobials

Hakan Kandemir [a,d], Cong Ma [b], Samuel K. Kutty [a], David StC. Black [a], Renate Griffith [c], Peter J. Lewis [b], Naresh Kumar [a,*]

[a] School of Chemistry, The University of New South Wales, Sydney, NSW 2052, Australia
[b] School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia
[c] School of Medical Sciences, The University of New South Wales, Sydney, NSW 2052, Australia
[d] School of Chemistry, Faculty of Art and Science, Namık Kemal University, Turkey

ARTICLE INFO

Article history:
Received 24 November 2013
Revised 9 January 2014
Accepted 17 January 2014
Available online 31 January 2014

Keywords:
Bis-indoles
Oxadiazoles
Thiadiazoles
Indole hydrazides
Antimicrobial
RNA polymerase

ABSTRACT

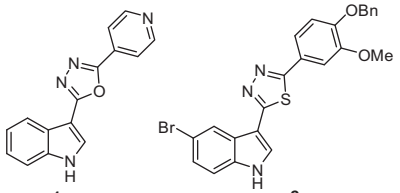
A range of novel hydrazine bridged bis-indoles was prepared from readily available indole-7-glyoxyloyl-chlorides and 7-trichloroacetylindoles and underwent cyclodehydration to produce 2,5-di(7-indolyl)-1,3,4-oxadiazoles and a 2,2′-bi-1,3,4-oxadiazolyl with phosphoryl chloride in ethyl acetate. This efficient protocol was subsequently used for the synthesis of 2- and 7-indolyl 2-(1,3,4-thiadiazolyl)ketones from related indolyl-hydrazine carbothioamides. The synthesised bis-indoles were evaluated for their antimicrobial properties, particularly the inhibition of protein–protein complex formation between RNA polymerase and σ factor and their bactericidal effect on Gram positive *Bacillus subtilis* and Gram negative *Escherichia coli*.

1. Introduction

The 1,3,4-oxadiazoles and 1,3,4-thiadiazoles are unique heterocyclic systems with importance in synthetic, medicinal and materials chemistry.[1] These five-membered heterocycles play a particularly vital role in medicinal chemistry because they display a variety of biological activities,[2–5] possess a favourable metabolic profile, and have a propensity to form hydrogen bonds. In particular, these ring systems have been found in marketed antihypertensive agents such as tiodazosin and nesapidil, antibiotics such as furamizole, and the carbonic anhydrase inhibitor acetazolamide.[6–9]

Indole linked oxadiazoles and thiadiazoles are novel classes of compounds that also exhibit a range of interesting biological activities. For example, the recently developed 2-(3-indolyl)-1,3,4-oxadiazole **1** has been screened as a potent anticancer agent and showed inhibitory activity against prostate and pancreatic cancer cell lines,[10] while the 2-(3-indolyl)-1,3,4-thiadiazole **2** displays significant cytotoxic activity against pancreatic cancer cell lines.[11]

There has been much recent interest in bis-indole amides and glyoxylamides as potent antibacterial agents. For example, compounds such as the 3-indolylglyoxylamide **3** have been shown to possess strong antibacterial activity against Gram-negative and Gram-positive bacteria.[12] We have also reported that a 7,7′-bis-indolylcarbohydrazide inhibit transcription initiation complex formation by preventing the unique bacterial σ initiation factor from binding to RNA polymerase.[13] It was therefore of interest to investigate the nature of the amide groups attached to the indole units and subsequent cyclisation of diamides and thiosemicarbazides to their bioisosters oxadiazoles and thiadiazoles respectively.



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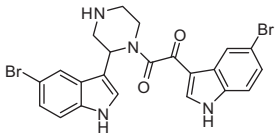

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* Corresponding author. Tel.: +61 2 9385 4698; fax: +61 2 9385 6141.
E-mail address: n.kumar@unsw.edu.au (N. Kumar).

http://dx.doi.org/10.1016/j.bmc.2014.01.025
0968-0896/

Given the importance of oxadiazoles and thiadiazoles, and the extensive scope available for the development of novel indole linked oxadiazoles and thiadiazoles, we were interested in the development of 2,5-di(7-indolyl)-1,3,4-oxadiazoles, and 2- and 7-indolyl 2-(1,3,4-thiadiazolyl)ketones. A range of synthetic approaches to 1,3,4-oxadiazoles have been reported in the literature, with many of these protocols involving the cyclization of acylhydrazides using harsh reagents such as thionyl chloride,[14] triflic anhydride[15] and phosphoryl chloride.[16] Mild cyclodehydration reagents such as Burgess reagent,[17] 4-methylbenzenesulfonyl chloride (TsCl)[18] and propylphosphonic anhydride (T3P)[19] have also been used. It was therefore anticipated that the target structures could be prepared via cyclization of indole-7-acylhydrazines, which in turn could be synthesized from methoxy activated indoles which are capable of undergoing reaction at the otherwise unreactive C7 position.[20,21]

2. Results and discussion

2.1. Synthesis of 2,5-di(7-indolyl)-1,3,4-oxadiazoles

The preparation of 2,5-di(7-indolyl)-1,3,4-oxadiazoles was achieved over a convenient three-step process. The first step involved the reaction of activated indoles **4a**–**c** with trichloroacetyl chloride at reflux for 3 h, which afforded 7-trichloroacetylindoles **5a**–**c** in 27–69% yields (Scheme 1).[22] Following this, 7-trichloroacetylindoles **5a**–**c** were heated at reflux for 24 h with half an equivalent of hydrazine hydrate in the presence of triethylamine in acetonitrile to afford the 7,7′-bis-indoles **6a**–**c** in 49–81% yield.

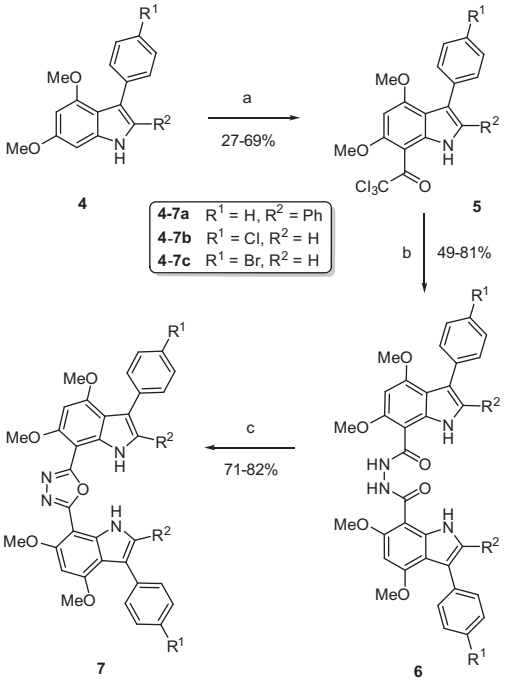
Initial attempts to generate the 2,5-di(7-indolyl)-1,3,4-oxadiazoles **7a**–**c** under mild conditions were unsuccessful. Heating bis-indoles **6a**–**c** at reflux with 1.1 equiv of T3P in the presence of triethylamine in ethyl acetate for 12 h resulted in recovery of the starting materials. Increasing the amount of T3P or base gave the same result. Similarly, no reaction was observed upon use of stronger bases such as DBU. It was therefore concluded that T3P was not strong enough to induce this dehydrative cyclization reaction.

The alternative stronger cyclodehydration reagent phosphoryl chloride was subsequently investigated. Treatment of bis-indoles **6a**–**c** with neat phosphoryl chloride at either room temperature or 50 °C resulted in formation of a black tar. Dilution of the reaction mixture with ethyl acetate as a solvent and heating bis-indoles **6a**–**c**at reflux for 2 h in the presence of excess phosphoryl chloride afforded the desired bis-indolyl-1,3,4-oxadiazoles **7a**–**c** in 71–82% yields after elimination of some baseline impurities by column chromatography.

Both bis-indoles **6a**–**c** and **7a**–**c** were poorly soluble in organic solvents, had high melting points and good stability under normal laboratory conditions. The ^{1}H NMR spectrum of the compound **7b** was characteristic for the oxadiazole compounds, showing the disappearance of hydrazide nitrogen protons of compound **6b** at 10.54 ppm, and a shift of the indole NH protons from 11.54 to 11.17 ppm. A high resolution mass spectrum further confirmed the structure, revealing the anticipated molecular ion.

2.2. Synthesis of 5,5′-di(7-indolyl)bi-2,2′-(1,3,4-oxadiazolyl) compounds

With the 2,5-di(7-indolyl)-1,3,4-oxadiazoles successfully in hand, it was of interest to extend the methodology to a related bi-2,2′-(1,3,4-oxadiazolyl) system **10**. Treatment of 7-trichloroacetylindole **5a** at room temperature for 1 h with 1.5 equiv of hydrazine hydrate in the presence of triethylamine gave the simple 7-carbohydrazide **8a** in high yield (Scheme 2).



Scheme 1. Reagents and conditions: (a) CCl_3COCl, 1,2-dichloroethane, reflux, 3 h; (b) $NH_2NH_2{\cdot}H_2O$, Et_3N, CH_3CN, reflux, 24 h; (c) $POCl_3$, EtOAc, reflux, 2 h.

Preparation of the symmetrical bis-oxalohydrazide **9a**, which possesses an extended linker between the two indole moieties, was subsequently carried out by addition of oxalyl chloride to hydrazide **8a** in dichloromethane at room temperature for 1 h.

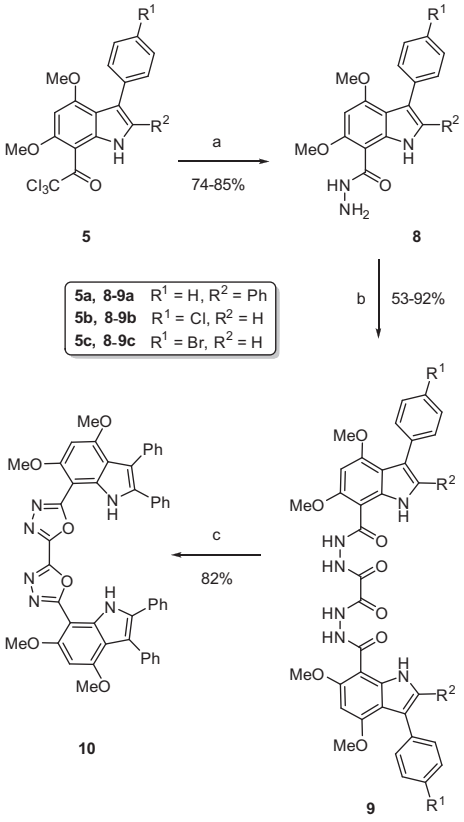
Triturating the crude product with hot methanol gave the pure compound **9a** in 53% yield.

The tandem cyclodehydration reaction was performed using the optimized conditions described above. Notably, a prolonged reaction time was required, with the reaction reaching completion after 24 h. Formation of bi-2,2′-(1,3,4-oxadiazolyl) **10** was indicated by the disappearance of the amide nitrogen protons of compound **9a** at 9.94 ppm in the ^{1}H NMR spectrum and a shift of the indole NH protons from 11.11 to 10.79 ppm. The presence of a molecular ion in the high resolution mass spectrum further confirmed the structure.

Similar treatment of 7-trichloroacetylindoles **5b** and **5c** afforded the corresponding indole-7-carbohydrazides **8b**,**c** and bis-oxalohydrazides **9b**,**c**. However, the dehydrative cyclization of these 3-substituted analogues proved to be problematic, resulting in a mixture of inseparable products.

The next objective of interest was the development of glyoxyl derivatives related to the di(7-indolyl)-1,3,4-oxadiazoles **7**. 4,6-Dimethoxyindole **4a** undergoes exclusive C7-substitution with oxalyl chloride to afford the 7-glyoxyloyl chloride **11** which can readily react with a range of amines to produce the corresponding glyoxyl amides.[23] Therefore, treatment of acid chloride **11** with a half equivalent of hydrazine hydrate in acetonitrile gave the bis-indole glyoxyloyl hyrazide **12** in 83% yield (Scheme 3).

Cyclodehydration of the bis-indole **12** was subsequently attempted under the optimized conditions, however, only recovery of the starting material was observed. The use of different solvents such as chloroform, dichloromethane, tetrahydrofuran and neat phosphoryl chloride at either room temperature or reflux, and the use of T3P in different solvents were examined but similarly gave no reaction. Two possible reasons causing the inhibition of the dehydrative cyclization in the 7,7′-linked system **12** are postulated. Firstly, there is potentially a greater steric restriction in the



Scheme 2. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, Et_3N, CH_3CN, rt, 1 h; (b) oxalyl chloride, Et_3N, DCM; rt, 1 h; (c) $POCl_3$, EtOAc, reflux, 24 h.

formation of the 1,3,4-oxadiazole due to the neighbouring glyoxyl carbonyl group. Alternatively, the strong hydrogen bonding between the carbonyl groups and NH protons could result in the formation of five or six membered rings which are too stable to be broken even under vigorous conditions.

2.3. Synthesis of 2- and 7-indolyl 2-(1,3,4-thiadiazolyl)ketones

With the apparent difficulties in achieving the cyclodehydration of the extended glyoxyl system **12**, attention turned to the simplified target system **15**. To the best of our knowledge, there are only limited reports relating to the preparation of indolyl-1,3,4-thiadiazoles or indolyl 2-(1,3,4-thiadiazolyl)ketones in the literature. Recently, the synthesis of 5-(3-indolyl)-1,3,4-thiadiazoles has been described.[11] It was anticipated that the indolyl 2-(1,3,4-oxothiadiazolyl)ketones could be synthesized through modification of the procedure developed for the 1,3,4-oxadiazoles described above. Reaction of 7-glyoxylchlorides **11** and **13** with 4,4-dimethyl-3-thiosemicarbazide in the presence of triethylamine in acetonitrile gave intermediates **14a** and **14b** in 75–79% yields (Scheme 4).

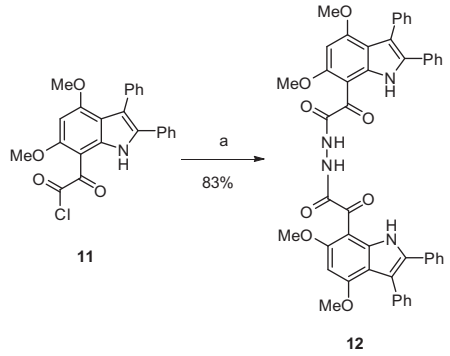
Subsequent cyclization of compounds **14a** and **14b** with phosphoryl chloride in ethyl acetate led to the formation of 7-indolyl 2-(1,3,4-thiadiazolyl)ketones **15a** and **15b** in 59% and 57% yields, respectively. The mass spectrum of the products **15a** and **15b** revealed molecular ions, while their ^1H NMR spectra showed the disappearance of the amide NH signals at 10.38 and 9.82 ppm from the respective precursors.

Similarly, the 2-indolylglyoxyloyl chlorides **16a** and **16b** reacted with 4,4-dimethyl-3-thiosemicarbazide to give 55% and 62% yields, respectively of the intermediates **17a** and **17b**, which were converted into the 2-indolyl-(1,3,4-thiadiazolyl) ketones **18a** and **18b** respectively in 33% and 36% yields (Scheme 5).

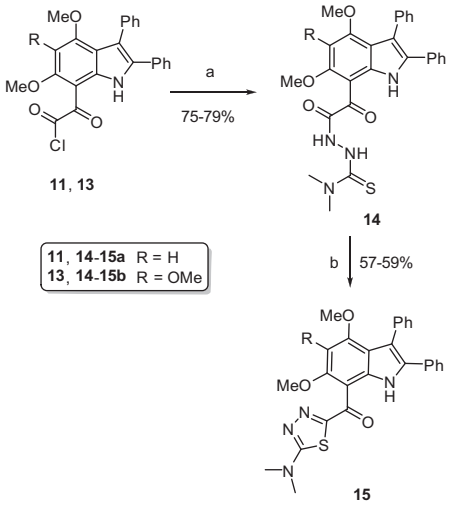
2.4. Biological properties of selected compounds

Significantly, in our previous study compound **6b** was found to inhibit the key protein–protein interaction between bacterial RNA polymerase (RNAP) and its cognate sigma factor at low micromolar concentration by specifically binding to the β′-CH region of the RNAP core.[13] Furthermore the peptidomimetic nature of the intermediate bis-amides **6**, **9** and **12** and their cyclic bioisosteres **7** and **10** makes them attractive for investigation as inhibitors of bacterial protein–protein interactions. Therefore, the synthesized compounds were evaluated for their biological properties particularly the inhibition of RNAP–σ initiation complex formation via an ELISA assay. Additionally, the antimicrobial properties of these compounds were tested against the Gram positive *Bacillus subtilis* and Gram negative *Escherichia coli* (Table 1). The 2,3-diphenylindole analogues were not tested due to their poor solubility in the assay conditions.

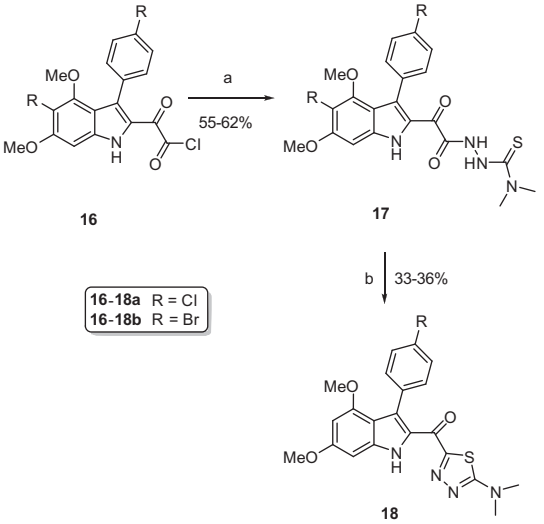
The results of the biology assay indicated that the bis-indolyl systems attached via diamides such as in compounds **6b**,**c** and indole thiosemicarbazides **17a**,**b** were most active in inhibiting RNAP-σ initiation complex formation and bacterial growth inhibition at late exponential phase. Particularly, compound **6b** was most active with 63% inhibition of RNAP-σ initiation complex formation via an ELISA assay and compound **17b** was most active in bacterial inhibition studies with 70% inhibition of both Gram positive *B. sub-*



Scheme 3. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, CH_3CN, rt, 1 h.



Scheme 4. Reagents and conditions: (a) 4,4-dimethyl-3-thiosemicarbazide, Et_3N, CH_3CN, rt, 1.5 h; (b) $POCl_3$, ethyl acetate, reflux, 2 h.



Scheme 5. Reagents and conditions: (a) 4,4-dimethyl-3-thiosemicarbazide, Et_3N, CH_3CN, rt, 1.5 h; (b) $POCl_3$, ethyl acetate, reflux, 2 h.

Table 1
Biological screening against RNAP-σ protein–protein interaction and bacterial growth inhibition

Compd	% In vitro binding inhibition ELISA (15 μM)	% In vivo bacterial growth inhibition at late exponential phase (200 μM)	
		B. subtilis (Gram positive)	*E. coli* (Gram negative)
6b	62.63	29.80	NA
6c	39.54	14.29	37.37
7b	17.83	NA	NA
7c	16.75	NA	NA
17a	20.44	32.48	46.96
17b	20.83	69.87	70.01
18a	17.23	NA	10.59
18b	18.98	NA	16.80

NA-no activity.

tilis and Gram negative *E. coli*. These results, in general, suggest that cyclisation of diamides **6** and thiosemicarbazides **17** to their bioisosteric oxadiazoles **7** and thiadiazoles **18** respectively decreases protein–protein inhibition activity. This trend in activity suggests that the need for hydrogen bond donors and a degree of flexibility in the linker are crucial for activity. It is also interesting to note that compounds **6b**,**c** have high in vitro (ELISA) inhibition but low in vivo inhibition activity, whereas compound **17b** has high in vivo inhibition activity but low in vitro (ELISA) inhibition. This could be attributed to low cell permeation of **6b**,**c** and possible non-specific interaction with other cellular targets of **17b**. Further structural modification and biological studies will be required to establish the correlation between the ELISA binding assay and the bacterial inhibition assay of these bis-indolyl and indole-thiosemicarbazide systems.

3. Conclusion

An efficient methodology for the synthesis of 2,5-di(7-indolyl)-1,3,4-oxadiazoles was developed and the tandem ring closure of a di(7-indolyl)-oxalohydrazide to a 5,5′-di(7-indolyl)-bi-2,2′-(1,3,4-oxadiazolyl) compound was achieved for a 2,3-diphenyl-substituted indole, but was not general. Adaptation of this synthetic methodology also led to the successful synthesis of monomeric 2- and 7-indolyl 2-(1,3,4-thiadiazolyl)ketones. The peptidomimetic indolyl bis-amides and indole-thiosemicarbazides showed promising antimicrobial properties. The cyclised derivatives tend to show decreased activity and this highlights the need for flexible linkers in order to retain the protein-protein inhibition activity. These indolyl peptidomimetics will be more biologically stable and considerably cheaper than synthesized peptides. Additionally, there is the potential for significant chemical modification to enhance their drug-like and antimicrobial properties (e.g. solubility, uptake). We are currently investigating the use of these compounds and the synthesis of further analogues as inhibitors of essential bacterial protein-protein interactions involved in nucleic acid synthesis and cell division that are unique to the eubacterial kingdom and historically have been underutilized for the development of clinically effective antibiotics.

4. Experimental

4.1. General methods

Melting points were measured using a Mel-Temp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Genesis Series FTIR spectrophotometer as KBr disks. Ultraviolet spectra were measured using a Varian Cary 100 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained in the designated solvents on a Bruker DPX 300. High-resolution mass spectrometry was performed by the Mass Spectrometry unit at the Bioanalytical Mass Spectrometry unit in the school of chemistry, UNSW. Microanalysis was performed on a Carlo Erba Elemental Analyzer EA 1108 at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. Anhydrous solvents were obtained using a PureSolv MD Solvent Purification System. Ajax Finechem Silica 200–325 mesh was used for column chromatography and Merck silica gel 60H was used for flash chromatography.

4.1.1. *N,N′*-Bis(4,6-dimethoxy-2,3-diphenyl-1*H*-indole-7-carbonyl) hydrazide (6a)

To a solution of 7-trichloroacetylindole **5a** (0.5 g, 1.05 mmol) in acetonitrile (20 mL), hydrazine hydrate (0.028 mL, 0.57 mmol) followed by triethylamine (5 drops) was added and the mixture was heated under reflux for 24 h. The resulting precipitate was filtered, washed with acetonitrile and water to afford the *title compound* (0.22 g, 57%) as a brown solid. Mp >320 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 3.80 (s, 6H, OMe), 4.19 (s, 6H, OMe), 6.28 (s, 2H, H5), 7.20–7.42 (m, 20H, aryl H), 10.94 (s, 2H, NH), 11.12 (br s, 2H, NH). IR (KBr): ν_{max} 3392, 2359, 1594, 1453, 1346, 1226, 1173, 1143, 991, 752, 699 cm^{-1}. UV–vis (CH_2Cl_2): λ_{max} 227 nm (ε 102,850 cm^{-1} M^{-1}), 250 (120,100), 323 (109,600). HRMS (+ESI): $C_{48}H_{38}N_4O_6$ $[M]^+$ requires 742.2791, found 742.2782. The sample was not soluble enough for ^{13}C NMR measurement.

4.1.2. *N,N′*-Bis(3-(4-chlorophenyl)-4,6-dimethoxy-1H-indole-7-carbonyl) hydrazide (6b)

To a solution of 7-trichloroacetylindole **5b** (0.372 g, 0.86 mmol) in acetonitrile (25 mL), hydrazine hydrate (0.021 mL, 0.43 mmol) followed by triethylamine (5 drops) was added and the mixture was heated under reflux for 24 h. The resulting precipitate was filtered, washed with acetonitrile and water to afford the *title compound* (0.23 g, 81%) as a brown solid. Mp >300 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 3.95 (s, 6H, OMe), 4.15 (s, 6H, OMe), 6.59 (s, 2H, H5), 7.28 (d, J = 2.1 Hz, 2H, H2), 7.41, 7.56 (2d, J = 8.5 Hz, 8H, aryl H), 10.54 (s, 2H, NH), 11.54 (br s, 2H, NH). IR (KBr): ν_{max} 3388, 1592, 1448, 1340, 1213, 1151, 792 cm^{-1}. UV–vis (THF): λ_{max} 243 nm (ε 86,634 cm^{-1} M^{-1}), 279 (45,830), 348 (57,198), 364 (42,599). HRMS (+ESI): $C_{34}H_{28}Cl_2N_4O_6$ $[M+Na]^+$ requires 681.1284, found 681.1273. The sample was not soluble enough for ^{13}C NMR measurement.

4.1.3. *N,N′*-Bis(3-(4-bromophenyl)-4,6-dimethoxy-1*H*-indole-7-carbonyl) hydrazide (6c)

To a solution of 7-trichloroacetylindole **5c** (0.6 g, 1.25 mmol) in acetonitrile (20 mL), hydrazine hydrate (0.03 mL, 0.43 mmol) followed by triethylamine (5 drops) was added and the mixture was heated under reflux for 24 h. The resulting precipitate was filtered, washed with acetonitrile and water to afford the *title compound* (0.23 g, 49%) as a pale brown solid. Mp >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.92 (s, 6H, OMe), 4.11 (s, 6H, OMe), 6.56 (s, 2H, H5), 7.24 (d, J = 2.5 Hz, 2H, H2), 7.46, 7.50 (2d, J = 8.8 Hz, 8H, aryl H), 10.50 (s, 2H, NH), 11.51 (d, J = 2.3 Hz, 2H, NH). IR (KBr): ν_{max} 3389, 1589, 1448, 1340, 1212 cm^{-1}. UV–vis (THF): λ_{max} 242 nm (ε 65,200 cm^{-1} M^{-1}), 281 (34,450), 348 (42,600), 365 (31,550). HRMS (+ESI): $C_{34}H_{28}Br_2N_4O_6$ $[M+Na]^+$ requires 769.0273, found 769.0265. The sample was not soluble enough for ^{13}C NMR measurement.

4.1.4. 2,5-Di(4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)-1,3,4-oxadiazole (7a)

To a solution of bis-indole **6a** (0.11 g, 0.14 mmol) in ethyl acetate (30 mL), $POCl_3$ (15 mL) was added and the solution was heated under reflux for 2 h. The solvent was evaporated under reduced pressure and the remaining residue was quenched with water and made alkaline by the addition of 5 N NaOH (30 mL). The resulting precipitate was collected by filtration, washed with water, dried and recrystallised from methanol to afford the *title compound* (0.09 g, 82%) as a white solid. Mp >320 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 6H, OMe), 4.04 (s, 6H, OMe), 6.38 (s, 2H, H5), 7.24–7.45 (m, 20H, aryl H), 10.79 (br s, 2H, NH). IR (KBr): ν_{max} 3380, 3353, 1598, 1489, 1351, 1328, 1220, 1147, 692 cm^{-1}. UV–vis (CH_2Cl_2): λ_{max} 247 nm (ε 105,018 cm^{-1} M^{-1}), 343 (90,292), 380 (65,425). HRMS (+ESI): $C_{46}H_{36}N_4O_5$ $[M+H]^+$ requires 725.2764, found 725.2761. The sample was not soluble enough for ^{13}C NMR measurement.

4.1.5. 2,5-Di(3-(4-chlorophenyl)-4,6-dimethoxy-1H-indol-7-yl)-1,3,4-oxadiazole (7b)

To a solution of bis-indole **6b** (0.109 g, 0.16 mmol) in ethyl acetate (30 mL), $POCl_3$ (10 mL) was added and the solution was heated under reflux for 2 h. The solvent was evaporated under reduced pressure and the remaining residue was quenched with water and made alkaline by the addition of 5 N NaOH (30 mL). The resulting precipitate was collected by filtration, washed with water, dried and purified by flash chromatography using dichloromethane/ethyl acetate (90:10) as eluent to afford the *title compound* (0.079 g, 74%) as a pale yellow solid. Mp 303–304 °C. (Found: C, 62.32; H, 4.10; N, 8.35; $C_{34}H_{26}Cl_2N_4O_5$ 0.2 CH_2Cl_2 requires C, 62.38; H, 4.04; N, 8.51). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (s, 6H, OMe), 4.06 (s, 6H, OMe), 6.63 (s, 2H, H5), 7.37 (d, J = 2.5 Hz, 1H, H2), 7.40, 7.57 (2d, J = 8.6 Hz, 8H, aryl H), 11.17 (d, J = 2.5 Hz, 2H, NH). IR (KBr): ν_{max} 3352, 1596, 1411, 1332, 1214, 1149, 1084, 985 cm^{-1}. UV–vis (THF): λ_{max} 247 nm (ε 64,600 cm^{-1} M^{-1}), 282 (35,000), 358 (51,300), 375 (40,400). HRMS (+ESI): $C_{34}H_{26}Cl_2N_4O_5$ $[M+H]^+$ requires 641.1359, found 641.1348. The sample was not soluble enough for ^{13}C NMR measurement.

4.1.6. 2,5-Di(3-(4-bromophenyl)-4,6-dimethoxy-1*H*-indol-7-yl)-1,3,4-oxadiazole (7c)

To a solution of bis-indole **6c** (0.101 g, 0.13 mmol) in ethyl acetate (30 mL), $POCl_3$ (10 mL) was added and the solution was heated under reflux for 2 h. The solvent was evaporated under reduced pressure and the remaining residue was quenched with water and made alkaline by the addition of 5 N NaOH (30 mL). The resulting precipitate was collected by filtration, washed with water, dried and purified by flash chromatography using dichloromethane/ethyl acetate (90:10) as eluent to afford the *title compound* (0.068 g, 71%) as a brown solid. Mp 299–301 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (s, 6H, OMe), 4.07 (s, 6H, OMe), 6.63 (s, 2H, H5), 7.37 (d, J = 2.5 Hz, 1H, H2), 7.50, 7.53 (2d, J = 8.9 Hz, 8H, aryl H), 11.18 (d, J = 2.2 Hz, 2H, NH). IR (KBr): ν_{max} 3353, 1596, 1537, 1484, 1411, 1332, 1214, 1084, 985 cm^{-1}. UV–vis (THF): λ_{max} 239 nm (ε 56,900 cm^{-1} M^{-1}), 284 (30,900), 358 (43,800), 375 (34,550). HRMS (+ESI): $C_{34}H_{26}{}^{79/81}Br_2N_4O_5$ $[M+H]^+$ requires 731.0328, found 731.0324.The sample was not soluble enough for ^{13}C NMR measurement.

4.1.7. 4,6-Dimethoxy-2,3-diphenyl-1*H*-indole-7-carbohydrazide (8a)

To a solution of 7-trichloroacetylindole **5a** (1.06 g, 2.46 mmol) in anhydrous acetonitrile (50 mL), hydrazine hydrate (0.167 mL, 3.44 mmol) was added followed by triethylamine (5 drops) and the mixture was stirred at room temperature for 1 h. Water was added to quench the reaction, the resulting precipitate was filtered and recrystallised from ethanol to afford the *title compound* (0.72 g, 85%) as a light brown solid. Mp 224–226 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 3H, OMe), 4.04 (s, 3H, OMe), 6.23 (s, 1H, H5), 7.24–7.45 (m, 10H, aryl H), 9.09 (s, 1H, NH), 11.21 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.71, 57.12 (OMe), 87.82 (C5), 126.48, 127.52, 127.83, 128.15, 128.34, 128.50, 128.83, 131.90 (aryl CH), 96.24, 114.17, 114.22, 133.00, 133.61, 136.26, 138.78, 157.07, 158.21 (aryl C), 169.10 (C=O). IR (KBr): ν_{max} 3392, 1620, 1596, 1494, 1462, 1235, 1112, 991, 700 cm^{-1}. UV–vis (MeOH): λ_{max} 249 (36,050 cm^{-1} M^{-1}), 319 (23,300). HRMS (+ESI): $C_{23}H_{22}N_3O_3$ $[M+H]^+$ requires 388.1661, found 388.1656.

4.1.8. 3-(4-Chlorophenyl)-4,6-dimethoxy-1*H*-indole-7-carbohydrazide (8b)

To a solution of 7-trichloroacetylindole **5b** (1.06 g, 2.46 mmol) in anhydrous acetonitrile (50 mL), hydrazine hydrate (0.167 mL, 3.44 mmol) was added followed by triethylamine (5 drops) and the mixture was stirred at room temperature for 1 h. Water was added to quench the reaction, the resulting precipitate was filtered and recrystallised from methanol to yield the *title compound* (0.72 g, 85%) as a light brown solid. Mp 203–205 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.91 (s, 3H, OMe), 4.06 (s, 3H, OMe), 6.28 (s, 1H, H5), 7.16 (d, J = 2.4 Hz, 1H, H2), 7.33, 7.53 (2d, J = 8.5 Hz, 4H, aryl H), 9.08 (s, 1H, NH), 11.11 (br s, 2H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.64, 57.16 (OMe), 87.65 (C5), 122.48 (C2), 128.13, 131.12 (aryl CH), 96.55, 111.31, 117.21, 131.99, 134.72, 139.69, 157.17, 157.85 (aryl C), 168.96 (C=O). IR (KBr): ν_{max} 3372, 3347, 1621, 1590, 1462, 1346, 1214, 1093 cm^{-1}. UV–vis (MeOH): λ_{max} 238 (39,400 cm^{-1} M^{-1}), 309 (18,200). HRMS (+ESI): $C_{17}H_{16}ClN_3O_3$ $[M+H]^+$ requires 346.0958, found 346.0953.

4.1.9. 3-(4-Bromophenyl)-4,6-dimethoxy-1*H*-indole-7-carbohydrazide (8c)

A solution of 7-trichloroacetylindole **5c** (0.499 g, 1.05 mmol) in anhydrous acetonitrile (20 mL), hydrazine hydrate (0.064 mL, 1.31 mmol) was added followed by triethylamine (3 drops) and the mixture was stirred at room temperature for 1 h. Water was added to quench the reaction, the resulting precipitate was filtered and recrystallised from ethanol to afford the *title compound* (0.3 g, 74%) as a pale yellow solid. Mp 215–217 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 3H, OMe), 4.02 (s, 3H, OMe), 6.25 (s, 1H, H5), 7.12 (d, J = 2.4 Hz, 1H, H2), 7.43, 7.47 (2d, J = 8.9 Hz, 4H, aryl H), 9.04 (s, 1H, NH), 11.07 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.13, 56.67 (OMe), 87.19 (C5), 121.95 (C2), 130.56, 130.98 (aryl CH), 96.07, 110.78, 116.72, 119.63, 134.69, 139.21, 156.68, 157.35 (aryl C), 168.44 (C=O). IR (KBr): ν_{max} 3363, 3344, 1621, 1588, 1463, 1344, 1214, 1093 cm^{-1}. UV–vis (MeOH): λ_{max} 237 (30,100 cm^{-1} - M^{-1}), 306 (14,200). HRMS (+ESI): $C_{17}H_{16}{}^{79}BrN_3O_3$ $[M+Na]^+$ requires 412.0273, found 412.0267.

4.1.10. *N,N′*-Bis(4,6-dimethoxy-2,3-diphenyl-1*H*-indole-7-carbonyl)oxalohydrazide (9a)

Oxalyl chloride (0.04 mL, 0.46 mmol) in dry dichloromethane (5 mL) was added dropwise to a solution of 7-carbohydrazide **8a** (0.34 g, 0.87 mmol) in dry dichloromethane (10 mL) containing triethylamine (0.12 mL, 0.87 mmol). The reaction mixture stirred at room temperature for 1.5 h. The solvent was then evaporated and the residue was quenched with water. The resulting precipitate was filtered, dried and recrystallised from methanol to yield the *title compound* (0.191 g, 53%) as a pale yellow solid. Mp >300 °C. (Found: C, 68.65; H, 4.93; N, 9.91; $C_{48}H_{40}N_6O_8$. 0.2 CH_2Cl_2 requires C, 68.44; H, 4.81; N, 9.94). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.74 (s, 6H, OMe), 4.05 (s, 6H, OMe), 6.45 (s, 2H, H5), 7.21–7.28 (m, 20H, aryl H), 9.94 (s, 2H, NH), 11.11 (s, 2H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.86, 57.15 (OMe), 88.76 (C5), 126.67, 127.59, 127.72, 127.85, 128.98, 131.45 (aryl CH), 95.84, 112.99, 114.00, 132.30, 132.34, 135.85, 137.93, 157.34, 158.08 (aryl C), 158.95, 165.90 (C=O). IR (KBr): ν_{max} 3379, 1629, 1594, 1430, 1351, 1238, 1144, 696 cm^{-1}. UV–vis (THF): λ_{max} 248 (67,800 cm^{-1} M^{-1}), 332 (60,250). HRMS (+ESI): $C_{48}H_{40}N_6O_8$ $[M+H]^+$ requires 829.2986, found 829.2978.

4.1.11. *N,N′*-Bis(3-(4-chlorophenyl)-4,6-dimethoxy-1*H*-indole-7-carbonyl)oxalohydrazide (9b)

Oxalyl chloride (0.04 mL, 0.46 mmol) in dry dichloromethane (5 mL) was added dropwise to a solution of 7-carbohydrazide **8b** (0.21 g, 0.61 mmol) in dry dichloromethane (10 mL) containing triethylamine (0.084 mL, 0.61 mmol). The reaction mixture was stirred at room temperature for 1.5 h. The solvent was then evaporated and the residue was quenched with water. The resulting precipitate was filtered, dried and recrystallised from methanol to yield the *title compound* (0.208 g, 92%) as a pale yellow solid. Mp >300 °C. (Found: C, 56.49; H, 4.13; N, 10.92; $C_{36}H_{30}Cl_2N_6O_8$ 0.3 CH_2Cl_2 requires C, 56.55; H, 4.0; N, 10.9). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.89 (s, 6H, OMe), 4.05 (s, 6H, OMe), 6.49 (s, 2H, H5), 7.23 (d, J = 2.5 Hz, 2H, H2), 7.36 (d, J = 8.6 Hz, 4H, aryl H), 7.51 (d, J = 8.4 Hz, 4H, aryl H), 9.84 (br s, NH, 2H), 10.80 (br s, NH, 2H), 11.41 (d, J = 1.2 Hz, 2H, NH) ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.80, 57.19 (OMe), 88.37 (C5), 123.81 (C2), 127.88, 131.04 (aryl CH), 96.23, 110.41, 115.78, 130.43, 135.01, 138.59, 157.20, 157.67 (aryl C), 158.69, 165.39 (C=O). IR (KBr): ν_{max} 3396, 1623, 1591, 1454, 1347, 1215 cm^{-1}. UV–vis (THF): λ_{max} 241 (67,550 cm^{-1} M^{-1}), 281 (34,850), 340 (35,700). HRMS (+ESI): $C_{36}H_{30}Cl_2N_6O_8$ $[M+H]^+$ requires 745.1580, found 745.1572.

4.1.12. *N,N′*-Bis(3-(4-bromophenyl)-4,6-dimethoxy-1*H*-indole-7-carbonyl)oxalohydrazide (9c)

Oxalyl chloride (0.04 mL, 0.46 mmol) in dry dichloromethane (5 mL) was added dropwise to a solution of 7-carbohydrazide **8c** (0.202 g, 0.52 mmol) in dry dichloromethane (10 mL) containing triethylamine (0.07 mL, 0.52 mmol). The reaction mixture was stirred at room temperature for 1.5 h. The solvent was then evaporated and the residue was quenched with water. The resulting precipitate was filtered, dried and recrystallised from methanol to yield the *title compound* (0.18 g, 83%) as a pale yellow solid. Mp >300 °C. (Found: C, 50.66; H, 3.71; N, 9.75; $C_{36}H_{30}Br_2N_6O_8$ 0.3 CH_2Cl_2 requires C, 50.70; H, 3.59; N, 9.77). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.89 (s, 6H, OMe), 4.05 (s, 6H, OMe), 6.49 (s, 2H, H5), 7.23 (d, J = 2.3 Hz, 2H, H2), 7.45 (d, J = 8.77 Hz, 4H, aryl H), 7.50 (d, J = 9.1 Hz, 4H, aryl H), 9.84 (s, NH, 2H), 10.78 (br s, NH, 2H), 11.41 (d, J = 1.6 Hz, 2H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.80, 57.19 (OMe), 88.39 (C5), 123.80 (C2), 130.79, 131.42 (aryl CH), 96.23, 110.35, 115.80, 118.92, 135.39, 138.60, 157.20, 157.67 (aryl C), 158.79, 165.38 (C=O). IR (KBr): ν_{max} 3395, 1621, 1589, 1457, 1347, 1215 cm^{-1}. UV–vis (THF): λ_{max} 241 (60,900 cm^{-1} M^{-1}), 284 (31,750), 343 (33,150). HRMS (+ESI): $C_{36}H_{30}Br_2N_6O_8$ $[M+Na]^+$ requires 855.0390, found 855.0385.

4.1.13. 5,5′-Bis(4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)-2,2′-bi-(1,3,4-oxadiazolyl) (10)

To a solution of bis-indole **9a** (0.094 g, 0.11 mmol) in ethyl acetate (30 mL), $POCl_3$ (15 mL) was added and the solution was heated under reflux for 24 h. The solution was poured slowly into ice-water and made alkaline by the addition of 5 N NaOH (30 mL). The resulting precipitate was collected by filtration, washed with water, dried and recrystallised from methanol to afford the *title compound* (0.058 g, 64%) as a yellow solid. Mp >300 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 6H, OMe), 4.13 (s, 6H, OMe), 6.32 (s, 2H, H5), 7.26–7.43 (m, 20H, aryl H), 10.79 (br s, 2H, NH). IR (KBr): ν_{max} 1597, 1485, 1425, 1358, 1219, 1149, 698 cm^{-1}. UV–vis (THF): λ_{max} 229 nm (ε 68,000 cm^{-1} M^{-1}), 248 (78,750), 334 (79,400). HRMS (+ESI): $C_{48}H_{36}N_6O_6$ $[M+H]^+$ requires 793.2775, found 793.2771. The sample was not soluble enough for ^{13}C NMR measurement.

4.1.14. *N,N′*-Bis(4,6-dimethoxy-2,3-diphenyl-1*H*-indole-7-glyoxyloyl)hydrazide (12)

To a solution of 7-glyoxyloyl chloride **11** (0.504 g, 1.2 mmol) in anhydrous acetonitrile (30 mL), hydrazine hydrate (0.031 mL, 0.64 mmol) was added followed by triethylamine (10 drops) and the solution was stirred at room temperature for 1.5 h. The reaction was quenched with ice-water and the resulting precipitate was filtered, dried and recrystallised from ethanol to yield the *title compound* (0.503 g, 100%) as a yellow solid. Mp 284–286 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 6H, OMe), 4.02 (s, 6H, OMe), 6.49 (s, 2H, H5), 7.28–7.32 (m, 20H, aryl H), 10.64 (br s, 2H, NH), 11.01 (br s, 2H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.24, 57.33 (OMe), 89.14 (C5), 126.80, 127.77, 127.90, 128.19, 128.33, 128.46, 128.69, 128.82, 131.40 (aryl CH), 101.33, 112.76, 114.87, 132.03, 133.18, 135.51, 137.10, 161.85, 162.45 (aryl C), 166.81, 188.53 (C=O). IR (KBr): ν_{max} 3414, 1643, 1583, 1466, 1361, 1348, 1242, 1219, 1140, 756, 699 cm^{-1}. UV–vis (CH_2Cl_2): λ_{max} 230 nm (ε 83,200 cm^{-1} M^{-1}), 272 (73,100), 337 (52,700). HRMS (+ESI): $C_{48}H_{38}N_4O_8$ $[M+Na]^+$ requires 821.2587, found 821.2582.

4.1.15. *N,N*-Dimethyl(4,6-dimethoxy-2,3-diphenyl-1*H*-indole-7-glyoxyloyl))hydrazinecarbothioamide (14a)

To a solution of 7-glyoxyloyl chloride **11**[23] (1.03 g, 2.46 mmol) in acetonitrile (30 mL), 4,4-dimethyl-3-thiosemicarbazide (0.306 g, 2.56 mmol) was added followed by triethylamine (7 drops). The mixture was stirred at room temperature for 1.5 h after which water was added to quench the reaction. The resulting precipitate was filtered, dried and recrystallised from dichloromethane/*n*-hexane to afford the *title compound* (0.975 g, 79%) as a yellow solid. Mp 153–155 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.24 (s, 6H, Me), 3.77 (s, 3H, OMe), 3.92 (s, 3H, OMe), 6.41 (s, 1H, H5), 7.24–7.29 (m, 10H, aryl H), 9.33 (s, 1H, NH), 10.38 (s, 1H, NH), 10.91 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 41.25 (Me), 56.18, 57.51 (OMe), 89.14 (C5), 126.74, 127.68, 127.86, 128.33, 128.76, 131.40 (aryl CH), 101.47, 112.67, 114.80, 132.06, 133.07, 135.57, 137.14, 161.54, 162.29 (aryl C), 183.38 (C=S), 165.95, 188.79 (C=O). IR (KBr): ν_{max} 3421, 1675, 1588, 1387, 1360, 1320, 1219, 1162, 696 cm^{-1}. UV–vis (MeOH): λ_{max} 247 (27,250 cm^{-1} M^{-1}), 331 (13,250). HRMS (+ESI): $C_{27}H_{26}N_4O_4S$ $[M+H]^+$ requires 503.1753, found 503.1748.

4.1.16. *N,N*-Dimethyl(4,5,6-trimethoxy-2,3-diphenyl-1*H*-indole-7-glyoxyloyl))hydrazinecarbothioamide (14b)

To a solution of 7-glyoxyloyl chloride **13**[21] (0.185 g, 0.41 mmol) in acetonitrile (10 mL), 4,4-dimethyl-3-thiosemicarbazide (0.054 g, 0.45 mmol) was added followed by triethylamine (3 drops). The mixture was stirred at room temperature for 1.5 h after which water was added to quench the reaction. The resulting precipitate

was filtered, dried and recrystallised from dichloromethane/*n*-hexane to afford the *title compound* (0.165 g, 75%) as a yellow solid. Mp 180–182 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.37 (s, 6H, Me), 3.66 (s, 3H, OMe), 3.82 (s, 3H, OMe), 4.02 (s, 3H, OMe), 7.26–7.36 (m, 10H, aryl H), 8.21 (d, J = 7.1 Hz, 1H, NH), 9.82 (d, J = 6.4 Hz, 1H, NH), 10.28 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.76 (Me), 61.28, 61.31, 62.59 (OMe), 126.60, 127.73, 127.98, 128.58, 131.14 (aryl CH), 106.39, 114.74, 118.78, 131.78, 133.37, 134.97, 135.07, 139.25, 155.21, 156.22 (aryl C), 178.68 (C=S), 162.06, 188.02 (C=O). IR (KBr): ν_{max} 3427, 3188, 2936, 1685, 1623, 1572, 1448, 1378, 1324, 1260, 1052, 828, 764, 698 cm^{-1}. UV–vis (MeOH): λ_{max} 249 (51,350 cm^{-1} M^{-1}), 363 (21,550). HRMS (+ESI): $C_{28}H_{28}N_4O_5S$ $[M+H]^+$ requires 533.1858, found 533.1852.

4.1.17. (4,6-Dimethoxy-2,3-diphenyl-1*H*-indol-7-yl) (5-(dimethylamino)-1,3,4-thiadiazol-2-yl)ketone (15a)

To a solution of indole **14a** (0.121 g, 0.24 mmol) in ethyl acetate (15 mL), $POCl_3$ (4 mL) was added and the solution was heated under reflux for 2 h. The solvent was evaporated under reduced pressure and the remaining residue was quenched with water and made alkaline by the addition of 5 N NaOH (12 mL). The resulting precipitate was collected by filtration, washed with water, dried and purified by flash chromatography using dichloromethane/ethyl acetate (70:30) as eluent to afford the *title compound* (0.067 g, 59%) as a yellow solid. Mp 180–182 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.26 (s, 6H, Me), 3.79 (s, 3H, OMe), 3.87 (s, 3H, OMe), 6.25 (s, 1H, H5), 7.21–7.39 (m, 10H, aryl H), 10.05 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.54 (Me), 55.32, 57.08 (OMe), 89.02 (C5), 126.07, 127.08, 127.34, 127.82, 128.34, 131.28 (aryl CH), 113.24, 114.86, 132.26, 132.82, 135.49, 137.67, 159.33, 160.48, 161.06, 173.66 (aryl C), 183.74 (C=O). IR (KBr): ν_{max} 3390, 1566, 1452, 1287, 1237, 1219, 1147, 698 cm^{-1}. UV–vis (MeOH): λ_{max} 283 nm (ε 29,900 cm^{-1} M^{-1}), 329 (21,000). HRMS (+ESI): $C_{27}H_{24}N_4O_3S$ $[M]^+$ requires 484.1569, found 484.1564.

4.1.18. (4,5,6-Trimethoxy-2,3-diphenyl-1*H*-indol-7-yl) (5-(dimethylamino)-1,3,4-thiadiazol-2-yl)ketone (15b)

To a solution of indole **14b** (0.109 g, 0.2 mmol) in ethyl acetate (10 mL), $POCl_3$ (3 mL) was added and the solution was heated under reflux for 2 h. The solvent was evaporated under reduced pressure and the remaining residue was quenched with water and made alkaline by the addition of 5 N NaOH (10 mL). The resulting precipitate was collected by filtration, washed with water, dried and purified by flash chromatography using dichloromethane/ethyl acetate (70:30) as eluent to afford the *title compound* (0.06 g, 57%) as a yellow solid. Mp 116–118 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.29 (s, 6H, Me), 3.60 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.97 (s, 3H, OMe), 7.23–7.41 (m, 10H, aryl H), 9.61 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.63 (Me), 61.04, 61.17, 62.48 (OMe), 126.31, 127.39, 127.57, 127.89, 128.40, 131.10 (aryl CH), 109.06, 114.55, 118.61, 132.02, 132.87, 134.41, 135.26, 140.05, 153.28, 153.33, 159.18, 174.10 (aryl C), 184.83 (C=O). IR (KBr): ν_{max} 3497, 2359, 2341, 1558, 1465, 1281, 1102, 1049, 695 cm^{-1}. UV–vis (MeOH): λ_{max} 231 nm (ε 45,150 cm^{-1} M^{-1}), 318 (29,400). HRMS (+ESI): $C_{28}H_{26}N_4O_4S$ $[M+H]^+$ requires 515.1753, found 515.1744.

4.1.19. *N,N*-Dimethyl(3-(4-chlorophenyl)-4,6-dimethoxy-1*H*-indole-2-glyoxyloyl))hydrazinecarbothioamide (17a)

To a solution of 2-glyoxyloyl chloride **16a**[23] (0.535 g, 1.42 mmol) in acetonitrile (25 mL), 4,4-dimethyl-3-thiosemicarbazide (0.186 g, 1.56 mmol) was added followed by triethylamine (6 drops). The mixture was stirred at room temperature for 1.5 h after which water was added to quench the reaction. The resulting precipitate was filtered, dried and recrystallised from dichloromethane/*n*-hexane to afford the *title compound* (0.552 g, 55%) as a yellow solid. Mp 219–221 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.21 (s, 6H, Me), 3.58 (s, 3H, OMe), 3.79 (s, 3H, OMe), 6.15 (d, J = 1.9 Hz, 1H, H5), 6.65 (d, J = 1.9 Hz, 1H, H7), 7.36 (s, 4H, aryl H), 9.35 (br s, 1H, NH), 10.80 (br s, 1H, NH), 11.91 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 41.25 (Me), 55.57, 55.76 (OMe), 86.89 (C5), 93.98 (C7), 127.06, 132.89 (aryl CH), 112.67, 126.37, 126.69, 131.90, 133.57, 139.99, 156.30, 161.29 (aryl C), 182.96 (C=S), 163.13, 178.05 (C=O). IR (KBr): ν_{max} 3129, 1701, 1631, 1529, 1389, 1309, 1207, 809 cm^{-1}. UV–vis (THF): λ_{max} 226 nm (ε 27,150 cm^{-1} M^{-1}), 247 (25,500), 356 (13,950). HRMS (+ESI): $C_{21}H_{21}ClN_4O_4S$ $[M+H]^+$ requires 461.1050, found 461.1047.

4.1.20. *N,N*-Dimethyl(3-(4-bromophenyl)-4,6-dimethoxy-1*H*-indole-2-glyoxyloyl))hydrazinecarbothioamide (17b)

To a solution of 2-glyoxyloyl chloride **16b**[23] (0.606 g, 1.44 mmol) in acetonitrile (25 mL), 4,4-dimethyl-3-thiosemicarbazide (0.189 g, 1.58 mmol) was added followed by triethylamine (6 drops). The mixture was stirred at room temperature for 1.5 h after which water was added to quench the reaction. The resulting precipitate was filtered, dried and recrystallised from dichloromethane/*n*-hexane to afford the *title compound* (0.45 g, 62%) as a yellow solid. Mp 217–219 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.21 (s, 6H, Me), 3.58 (s, 3H, OMe), 3.79 (s, 3H, OMe), 6.15 (d, J = 1.9 Hz, 1H, H5), 6.65 (d, J = 1.9 Hz, 1H, H7), 7.30, 7.49 (2d, J = 8.2 Hz, 4H, aryl H), 9.35 (br s, 1H, NH), 10.80 (br s, 1H, NH), 11.92 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 41.22 (Me), 55.59, 55.76 (OMe), 86.89 (C5), 94.0 (C7), 129.97, 133.23 (aryl CH), 112.60, 126.37, 126.66, 133.97, 140.01, 156.30, 161.30 (aryl C), 182.94 (C=S), 163.12, 178.08 (C=O). IR (KBr): ν_{max} 3134, 1701, 1638, 1389, 1207, 1156, 809 cm^{-1}. UV–vis (THF): λ_{max} 226 nm (ε 35,850 cm^{-1} M^{-1}), 252 (32,250), 356 (17,150). HRMS (+ESI): $C_{21}H_{21}BrN_4O_4S$ $[M+H]^+$ requires 505.0545, found 505.0532.

4.1.21. (3-(4-Chlorophenyl)-4,6-dimethoxy-1*H*-indol-2-yl)(5-(dimethylamino)-1,3,4-thiadiazol-2-yl)ketone (18a)

To a solution of indole **17a** (0.186 g, 0.4 mmol) in ethyl acetate (20 mL), $POCl_3$ (3 mL) was added and the solution was heated under reflux for 2 h. The solution was poured slowly into ice–water (40 mL) and made alkaline by the addition of 5 N NaOH (10 mL). It was then extracted with ethyl acetate (2 × 20 mL) and dichloromethane (1 × 20 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude mixture was purified by flash chromatography using dichloromethane/ethyl acetate (90:10) as eluent to afford the *title compound* (0.057 g, 33%) as an orange-brown solid. Mp 276–278 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27 (s. 6H, Me), 3.64 (s, 3H, OMe), 3.87 (s, 3H, OMe), 6.10 (d, J = 1.9 Hz, 1H, H5), 6.43 (d, J = 1.9 Hz, 1H, H7), 7.34, 7.44 (2d, J = 8.7 Hz, 4H, aryl H), 11.50 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.56 (Me), 55.04, 55.46 (OMe), 85.77 (C5), 93.35 (C7), 126.97, 131.98 (aryl CH), 113.07, 126.85, 127.68, 132.74, 133.13, 139.41, 156.64, 161.44 (aryl C), 162.72 (N–C=N), 170.54 (C=O–C=N), 174.03 (C=O). IR (KBr): ν_{max} 3343, 1611, 1484, 1290, 1203, 1154, 1047, 921 cm^{-1}. UV–vis (THF): λ_{max} 226 nm (ε 45,400 cm^{-1} M^{-1}), 337 (16,150). HRMS (+ESI): $C_{21}H_{19}ClN_4O_3S$ $[M+H]^+$ requires 443.0945, found 443.0936.

4.1.22. (3-(4-Bromophenyl)-4,6-dimethoxy-1*H*-indol-2-yl)(5-(dimethylamino)-1,3,4-thiadiazol-2-yl)ketone (18b)

To a solution of indole **17b** (0.45 g, 0.89 mmol) in ethyl acetate (20 mL), $POCl_3$ (3 mL) was added and the solution was heated under reflux for 2 h. The solution was poured slowly into ice–water (40 mL) and made alkaline by the addition of 5 N NaOH (10 mL). It was then extracted with ethyl acetate (2 × 20 mL) and dichloromethane (1 × 20 mL). The organic extracts were combined, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude mixture was purified by flash chromatography

using dichloromethane/ethyl acetate (90:10) as eluent to afford the *title compound* (0.153 g, 36%) as an orange-brown solid. Mp 280–282 °C. (Found: C, 51.28; H, 3.87; N, 11.23; $C_{21}H_{19}BrN_4O_3S0.1\ CH_2Cl_2$ requires C, 51.11; H, 3.90; N, 11.30). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.20 (s. 6H, Me), 3.57 (s, 3H, OMe), 3.79 (s, 3H, OMe), 6.14 (d, J = 1.9 Hz, 1H, H5), 6.84 (d, J = 1.9 Hz, 1H, H7), 7.27, 7.45 (2d, J = 8.8 Hz, 4H, aryl H), 11.83 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 41.66 (Me), 55.28, 55.54 (OMe), 87.62 (C5), 93.65 (C7), 129.88, 133.26 (aryl CH), 112.40, 120.18, 125.61, 127.06, 134.68, 139.84, 156.08 (aryl C), 160.69 (N–C=N), 172.39 (C=O–C=N), 173.95 (C=O). IR (KBr): ν_{max} 1610, 1540, 1456, 1291, 1242, 1203, 1154, 813 cm^{-1}. UV–vis (THF): λ_{max} 292 nm (ε 31,350 cm^{-1}-M^{-1}), 329 (13,350). HRMS (+ESI): $C_{21}H_{19}{}^{81}BrN_4O_3S$ $[M+H]^+$ requires 489.0374, found 489.0408.

4.2. ELISA based assay

Purified full-length σ^A was diluted to 250 nM in phosphate buffered saline (PBS) and 100 μL of the solution added into NUNC Maxisorp™ microtitre plate wells. Following overnight incubation at 4 °C the wells were washed 3 times with 300 μL of PBS and blocked with 300 μL of 1% (w/v) BSA in PBS at room temperature for 2 h. After blocking, plates were washed three times with wash buffer (PBS, 0.05% (v/v) Tween-20). 200 nM wild-type or mutant GST tagged β′ CH region in 100 μL PBS was then added to wells followed by incubation for 1 h at room temperature. Wells were washed 3 times in 300 μL of PBS/Tween-20 wash buffer before the addition of 100 μL rabbit anti-GST primary antibody (1:2000 in PBS) to each well and incubated for 1 hour at room temperature. Next, HRP-conjugated goat-anti-rabbit secondary antibody (1:2000 in PBS) was added to each well and incubated for 1 h at room temperature, with three 300 μL PBS/Tween-20 washes between each of the antibody incubations. Interactions were detected by the addition of 100 μL TMB substrate system (3,3′,5,5′-Tetramethylbenzidine liquid substrate system for ELISA, Sigma–Aldrich) to each well. The plate was then incubated with shaking at 600 rpm in a FLUOstar Optima plate reader (BMG Labtech) at room temperature for 6 min prior to measurement of the absorbance at 600 nm.

4.3. In vivo activity test

The compounds were dissolved to 50 mM in DMSO and diluted in 100 μL of LB medium to 200 μM in 96-well NUNC Microwell™ plate. *B. subtilis* 168 was used as the Gram positive and *E. coli* DH5α as the Gram negative test cells. The cells were grown at 37 °C in 5 mL LB with shaking until the OD reached 0.6–0.7, and 5 μL of the culture was added to each well. The plate was incubated in the FLUOstar Optima plate reader (BMG Labtech) at 37 °C with 600 rpm shaking. The OD of the culture was taken every 10 min using LB as the blank for 16 h at 600 nm. The samples were tested in triplicate and the growth pattern of each sample was compared to cells exposed to equal amounts of DMSO.

Acknowledgments

We thank the University of New South Wales and the Turkish Government for their financial support. This work was supported with funding from the NHMRC (APP1008014).

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