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Synthesis and Biological Activity of Novel Bis–Indole Inhibitors of Bacterial Transcription Initiation Complex Formation

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The increasing resistance of bacteria against clinically approved antibiotics is resulting in an alarming decrease in therapeutic options for today's clinicians. We have targeted the essential interaction between bacterial RNA polymerase and σ^{70}/σ^{A} for the development of lead molecules exhibiting a novel mechanism of antibacterial activity. Several classes of structurally related bis–indole inhibitors of bacterial transcription initiation- complex formation were synthesized and their antimicrobial activities were evaluated. Condensation of indole–7– and indole–2–carbohydrazides with 7– and 2–trichloroacetylindoles or indole–7– and indole–2–glyoxyloyl chlorides resulted in the successful synthesis of 7,7′–, 2,2′–, 2,7′– and 3,2′–linked bis–indole derivatives with –CO–NH–NH–CO– and –CO–CO–NH–NH–CO–linkers. Indole–7–glyoxyloyl chlorides were reacted with hydrazine hydrate in different ratios to afford respective –CO–CO–NH–NH–CO–CO– bis–indole or hydrazide derivatives. The resulting compounds were found to be active against the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction in ELISA assays and inhibited the growth of both Gram–positive and Gram–negative bacteria. Structure–activity relationship (SAR) studies were performed in order to identify the structural features of the synthesized inhibitors required for biological activity.

Introduction

Since Alexander Fleming's serendipitous discovery of the antibacterial activity of the fungus *Penicillium notatum* in 1928, antibiotics have emerged as the primary treatment option for infectious diseases.[1] Unfortunately, the over–use of antibiotics has resulted in the rapid development of drug–resistant bacterial strains.[2-6] Most of the antibiotic classes in common use today were discovered between 1945 and 1960, during the "Golden Era" of antibiotics, but since that time only a few more new classes of antibiotics have been developed.[6] Moreover, the discovery and development of new antibacterial agents has decreased at an alarming rate in recent years.[1,7] In the absence of new antibacterial drugs, antibiotic resistance threatens to pose a grave threat to human health in the coming years.[8,9] Compounding this problem, many new compounds being released to the market are derivatives of existing drugs to which resistance has already appeared. Consequently, there is a clear and urgent need for the discovery and development of novel classes of antibiotics and drug targets that can slow the rate of appearance of resistance.[6]

Bacterial RNA polymerase (RNAP) and transcription are promising targets that have been exploited for the development of novel antimicrobials. There are two forms of bacterial RNAP called core and holoenzyme, and transcription consists of three main sequential stages: initiation, elongation and termination.[10] The RNAP core enzyme is catalytically active, but is unable to initiate transcription with appropriate specificity and efficiency when transcription initiation factor σ is not present.[10,11] In order to form a holoenzyme recognizing DNA promoters and initiating the transcription cycle, the core enzyme must associate with σ factors.[10,12] σ Factors are unique to bacteria and are essential for correct gene expression and viability, with the σ^{70} factor in Gram–negative bacteria and the σ^{A} factor in Gram–positive bacteria being the most important proteins required for direct contact between RNAP and promoter DNA sequences.[11-14] The interaction between region 2.2 of σ^{70}/σ^{A} and the β'–CH region of the RNAP core is essential for the formation of the RNAP holoenzyme.[15] Domains of σ^{70}/σ^{A} factors that interact with the core enzyme are highly conserved,[10,12,16,17] and so molecules capable of disrupting the interaction between σ^{70}/σ^{A} and the core enzyme would be

expected to exhibit broad spectrum activity. Therefore, the interaction between $\sigma^{70}/\sigma^{A}{}_{2.2}$ and the β'–CH region represents an excellent target for the design and development of new antibiotic leads with novel scaffolds and a completely unique mechanism of antimicrobial activity.

There are numerous natural and synthetic compounds that inhibit transcription by targeting RNAP.[12,18-22] Rifampicin and lipiarmycin target the active site of RNAP and are especially active against Gram–positive bacteria, such as *Mycobacterium tuberculosis* and *Clostridium difficile*, but resistance can rapidly develop through mutation at multiple locations within the active site.[12,23] Sorangicin and streptolydigin, antibiotics not approved in the clinic, also exhibit selective activity against Gram–positive bacteria.[12,24] Recently, smaller molecules such as SB2, SB4, SB5, SB7, SB8 and SB12 have been successfully identified as potential inhibitors of the interaction between σ^{70}/σ^{A} and RNAP.[19]

As the majority of the antibiotics targeting transcription exhibit significant activity against only Gram–positive bacteria, novel compounds to combat Gram–negative bacteria are urgently required due to the rapid increase in resistant strains.[12,23]

In our preliminary research, bis–indole compounds **GKL001 (16)** and **GKL003 (10a)** (Figure 1) were found to inhibit the essential interaction between $\sigma^{70}/\sigma^{A}{}_{2.2}$ and the β'–CH region of the core RNAP in ELISA assays. Moreover, modeling studies employing a *Bacillus subtilis* RNAP homology model[25] and isothermal titration calorimetry experiments[26] indicated that **GKL003** binds to the β'–CH region of RNAP, thereby inhibiting transcription initiation. Both molecules inhibited the growth of *Bacillus subtilis* and *Escherichia coli*.

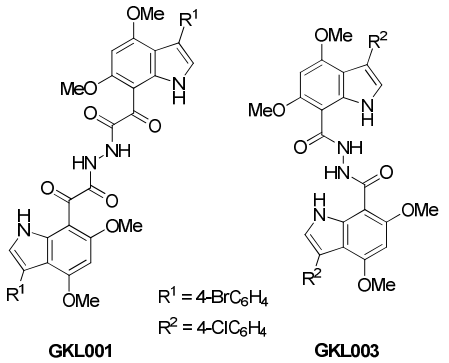


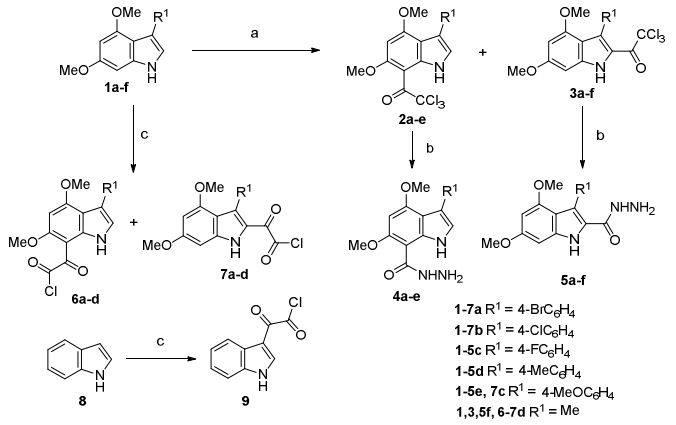
Figure 1. Bis–indoles as potential inhibitors of interaction between RNAP core and σ^{70}/σ^{A} in bacteria.

The aim of this study was to elaborate our bis–indole library and to develop novel inhibitors of the interaction between RNAP and σ^{70}/σ^{A}, and to evaluate the antibacterial activity of the compounds by ELISA and bacterial growth inhibition assay. In this paper, we report the discovery of molecules that act against both Gram–positive and Gram–negative bacteria, and present our structure–activity relationship studies (SAR) on this class of novel RNAP–σ^{70}/σ^{A} inhibitors.

Results and discussion

Variation of substituents at position 3 of the indole ring, types of linkage between the two indole units (7,7′, 2,2′, 2,7′ and 3,2′) and types of linkers (–CO–NH–NH–CO–, –CO–CO–NH–NH–CO– and –CO–CO–NH–NH–CO–CO–) were considered to produce analogues of **GKL001 (16)** and **GKL003 (10a)**.

The well–established five–step synthesis of 3–aryl–4,6–dimethoxyindoles **1a–e** and the one–pot synthesis of 4,6–dimethoxy–3–methylindole **1f** (Scheme 1) were previously reported by our group.[27] Owing to the nature of the activating substituents, 3–aryl–4,6–dimethoxyindoles **1a–e** and 4,6–dimethoxy–3–methylindole **1f** are able to undergo electrophilic substitution reactions without the use of a catalyst. Therefore, indoles **1a–f** were reacted two reagents, trichloroacetyl chloride or oxalyl chloride to yield varieties of indoles such as 7–trichloroacetylindoles **2a–e**, 2–trichloroacetylindoles **3a–f** , indole–7–glyoxyloylchlorides **6a–d** and indole–2–glyoxyloyl chlorides **7a–d**,[28] respectively (Scheme 1).

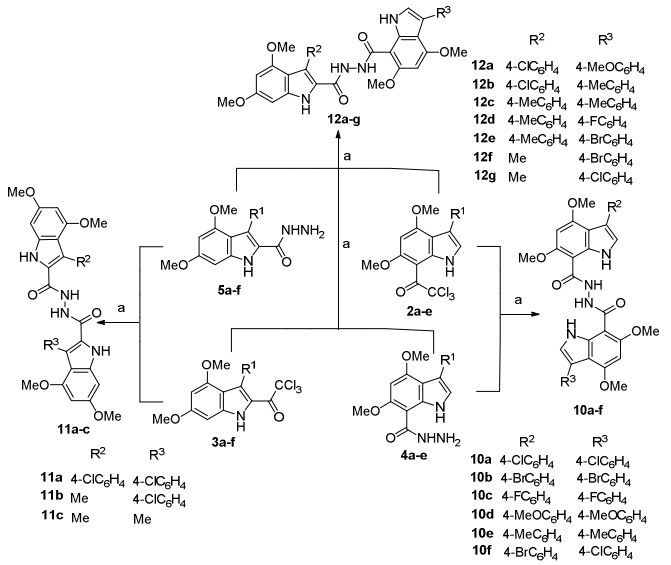


Scheme 1. Reagents and conditions: (a) CCl_3COCl (3 equiv), 1,2–dichloroethane, 80 °C, 3.5 h, 20–37% **(2a–e)**, 10–23% **(3a–f)**; (b) $NH_2NH_2{\cdot}H_2O$ (5–9 equiv), MeCN, room temp., 3.5 h, 74–80% **(4a–e)**, 73–81% **(5a–f)**; (c) oxalyl chloride (3 equiv), diethyl ether, 0 °C → room temp., 3 h, 30–33% **(6a–d)**, 35–43% **(7a–d)**.

Anhydrous 1,2–dichloroethane was found to be the most suitable solvent for the reaction between indoles **1a–f** and trichloroacetyl chloride, as it provided reasonable yields of the products (20–37% for the 7–trichloroacetyl derivatives **2a–e** and 10–23% for the 2–trichloroacetylderivatives **3a–f** after chromatographic separation) and minimal production of a third isomeric side product, *N*–trichloroacetylindole (<1%). Interestingly, the 7–trichloroacetyl derivatives were not produced when 4,6–dimethoxy–3–methylindole was reacted with trichloroacetyl chloride. Treatment of a suspension of the trichloroacetyl derivatives **2a–e** and **3a–f** in acetonitrile with excess hydrazine hydrate (5–9 equivalents) resulted in formation of the corresponding indole–7–carbohydrazides **4a–e** and indole–2–carbohydrazides **5a–f** in very good yields (74–80% and 73–81%, respectively) and with no chromatography required. The indoles **1a–f** were found to be

more reactive with oxalyl chloride compared to trichloroacetyl chloride, and so the reactions could be performed in diethyl ether at 0 °C[28] in order to minimize formation of side products. Similarly, two isomeric products were obtained: indole–7–glyoxyloyl chlorides **6a–d** (30–33%) and indole–2–glyoxyloyl chlorides **7a–d** (35–43%). Following a well–established procedure, indole–3–glyoxyloyl chloride **9** was synthesized from indole **8**.[29]

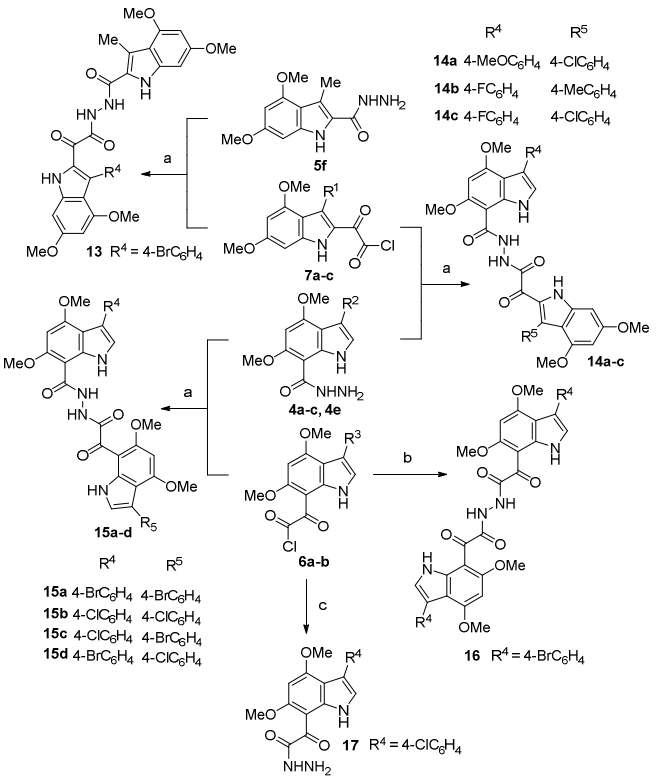
Condensations of the trichloroacetyl derivatives **2a–e** and **3a–f** with the hydrazides derivatives **4a–e** and **5a–f** in anhydrous acetonitrile with triethylamine as a catalyst resulted in the formation of a variety of novel bis–indoles **10a–f**, **11a–c** and **12a–g** comprising the –CO–NH–NH–CO– motif (Scheme 2). Both symmetrical and unsymmetrical molecules were synthesized using three different types of linkages: the 7,7′ linkage **(10a–f)**, the 2,2′ linkage **(11a–c)** and the 2,7′ linkage **(12a–g)**. Bis–indoles **12a–g** could be obtained from the reaction of the trichloroacetyl derivatives **2a–e** with hydrazides **5a–f**, or from the reaction of the trichloroacetyl derivatives **3a–f** with hydrazides **4a–e**, based on the availability of the starting materials. Depending on the reactivity of the building blocks **2a–e**, **3a–f**, **4a–e** and **5a–f**, reactions were carried out at room temperature or at reflux for 5–24 h.



Scheme 2. Reagents and conditions: (a) Et_3N, MeCN, room temp. or reflux, 5–24 h, 40–81%.

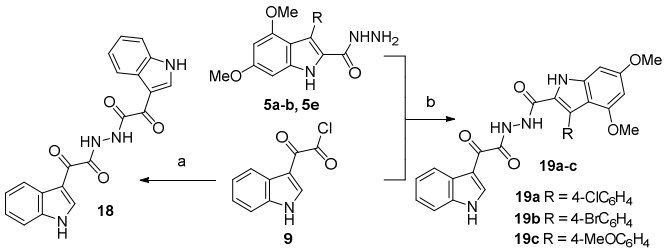
When glyoxyloyl chlorides **6a–b** and **7a–c** were condensed with hydrazides **4a–c**, **4e** and **5f**, 7,7′–linked bis–indoles **15a–d**, 2,2′–linked bis–indole **13** and 2,7′–linked bis–indoles **14a–c** containing an unsymmetrical –CO–CO–NH–NH–CO– linker were produced (Scheme 3). Because glyoxyloyl chlorides were found to decompose at higher temperatures and were readily susceptible to hydrolysis into glyoxylic acids in the presence of moisture, all reactions were carried out at room temperature in anhydrous acetonitrile with triethylamine as a catalyst.

Two different products were obtained when glyoxyloyl chlorides **6a–b** were reacted with hydrazine hydrate. When a 2:1 molar ratio of the glyoxyloyl chloride **6a** to hydrazine hydrate was used, the symmetrical bis–indole **16** was produced. However, when excess hydrazine hydrate was used in the glyoxyloyl chloride **6b**, the hydrazide **17** was generated instead.



Scheme 3. Reagents and conditions: (a) Et_3N, MeCN, room temp., 5–26 h; (b) $NH_2NH_2{\cdot}H_2O$ (0.5 equiv), Et_3N, MeCN, room temp., 1.5 h; (c) $NH_2NH_2{\cdot}H_2O$ (excess), Et_3N, MeCN, room temp., 5 h.

Similarly, when 3–glyoxyloyl chloride **9** was reacted with hydrazine hydrate in a 2:1 molar ratio, the symmetrical 3,3′–linked bis–indole **18** was produced.[30] In addition, when glyoxyloyl chloride **9** and hydrazides **5a–b** and **5e** were reacted, it produced 3,2′–linked bis–indoles **19a–c** (Scheme 4).



Scheme 4. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$ (0.5 equiv), Et_3N, MeCN, room temp., 1.5 h; (b) Et_3N, MeCN, room temp., 5 h.

A library of thirty synthesized compounds was evaluated for antibacterial activity. Inhibition of the interaction between $\sigma^{70}/\sigma^{A}{}_{2.2}$ and the β'–CH region of the core RNAP was examined by ELISA at 15 μM compound concentration and expressed as a % of the negative control. Bacterial growth inhibition was evaluated at *ca.* 200 μM compound concentration using two

representative bacterial species, *B.subtilis* (Gram–positive) and *E. coli* (Gram–negative), and expressed as a % of the negative control (Table 1). Negative control was related to either the interaction between $\sigma^{70}/\sigma^{A}{}_{2.2}$ and the β'–CH region of the core RNAP or bacterial growth in the absence of the inhibitor of transcription initiation complex formation.

Table 1. Evaluation of antibacterial activity of the synthesized compounds

Compound	β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ binding inhibition at 15 μM by ELISA [%]	Bacterial growth inhibition at 200 μM [%]	
		Bacillus subtilis	*Escherichia coli*
10a	63	30[a]	ND
10b	40	14[a]	37[a]
10d	73	13[a]	23[a]
10e	68	11[a]	21[a]
10f	60	No activity[a]	43[a]
11b	50	No activity	16
11c	86	No activity	16
12a	62	No activity[a]	21[a]
12b	58	No activity[a]	No activity[a]
12d	60	No activity[a]	No activity[a]
12f	61	No activity	23
12g	72	No activity	45[b]
13	66	No activity	16
14a	71	No activity[a]	25[a]
14b	72	No activity[a]	23[a]
14c	61	No activity[a]	23[a]
15a	72	No activity[a]	No activity[a]
15b	63	No activity[a]	6[a]
15c	52	No activity[a]	35[a]
15d	68	No activity[a]	22[a]
16	39	31[a,b]	15[a]
17	25	87[a,b]	No activity[a]
18	50	47[b]	33
19a	57	25[b]	15
19b	16	3	11
19c	41	No activity	39

[a] precipitation at *ca.* 200 μM, [b] affects exponential phase of bacterial growth, ND no data

The compounds generally showed significant activity against the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ binding interaction at 15 μM, with 21 of the 30 novel compounds exhibiting ≥50% inhibition at this concentration. Surprisingly, the molecules were found to be more active against Gram–negative bacteria than Gram–positive bacteria, with 14 of the 30 molecules exclusively inhibiting *E. coli* growth at *ca.* 200 μM compound concentration. Compounds **10f**, **12g**, **15c** and **19c** showed ≥35% exclusive inhibition of *E. coli* growth. Moreover, molecule **12g** being the most potent inhibitor of *E. coli* growth, was found to affect exponential phase of bacterial growth (Table 1). Exponential phase of bacterial growth is a phase of growth observed in a bacterial population where the growth of cells increases by a multiplicative factor per unit of time. Compound **18** could be a good broad spectrum candidate as it exhibited 47% inhibition of *B. subtilis* growth and 33% inhibition of *E. coli* growth, respectively. Many of the molecules precipitated out of the solution when diluted to *ca.* 200 μM concentration with the media (Table 1).

Based on the biological activity of the synthesized compounds, structure–activity relationship (SAR) studies could be performed. The compounds synthesized in this study varied in the nature of the linkage between the two indole units and in the type of substituent at position 3 of the indole ring. Three different types of linkers (–CO–CO–NH–NH–CO–CO–, –CO–CO–NH–NH–CO– and –CO–NH–NH–CO–) in combination with four types of linkage (7,7′, 2,2′, 2,7′ and 3,2′) were represented in the library of compounds.

For 7,7′–linked compounds with a –CO–NH–NH–CO– linkage compound **10a** with a 4–ClC_6H_4 substituent at position 3 of the indole rings showed 63% inhibition of the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction at 15 μM, compared to 40% for **10b** containing a 4–BrC_6H_4 substituent. Furthermore, **10a** was over twice as potent against *B. subtilis* growth compared to **10b**. Interestingly, when the7–CO–NH–NH–CO–7′ linkage **10b** was replaced by the 7–CO–CO–NH–NH–CO–CO–7′ linkage **16**, a two–fold increase in inhibition of *B. subtilis* growth was observed, which was accompanied by a more than two–fold decrease in inhibition of *E. coli* growth. However, there was no significant difference in the ability of the compounds to inhibit the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction. This suggested that the variation in antibacterial activity of **10b** and **16** in the two types of bacteria could be due to differences in permeability, since Gram–positive and Gram–negative bacteria have substantial differences in the nature of their cell walls. Interestingly, incorporation of the 7–CO–CO–NH–NH–CO–7′ linkage **15a–d** completely abolished the ability of the molecules to inhibit *B. subtilis* growth, but had no effect on the ability of these molecules to inhibit the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction. These differences might be due to the ability of these compounds to cross cell walls and membranes of the Gram–positive and Gram–negative bacteria. Notably, molecules **10f** and **15b–d** showed activity against the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction and were also exclusive inhibitors of *E. coli* growth. Good correlation between inhibition of the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction and inhibition of *E. coli* growth for molecules **10f** and **15c** suggested that inhibition of the interaction between $\sigma^{70}/\sigma^{A}{}_{2.2}$ and the β'–CH region of the core RNAP is the mechanism of observed antibacterial activity.

Compounds with 2,2′ linkage **(11b–c** and **13)** showed low inhibition of *E. coli* growth. Moreover, they did not inhibit *B. subtilis* growth regardless of the type of linkage and the nature of the substituent at position 3 of the indole ring. Given that these molecules exhibited relatively high activities against the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction, their low antibacterial activity against *E. coli* and no activity against *B. subtilis* might be due to their limited ability to cross the outer membrane of the cell wall in Gram–negative bacteria and the cell wall in Gram–positive bacteria.

The 2,7′–linked compounds were found not to inhibit *B. subtilis* growth. An increase in β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ inhibitory activity accompanied by a two–fold increase in antibacterial activity against *E. coli* was observed when a 4–BrC_6H_4 substituent **12f** was replaced with a 4–ClC_6H_4 substituent **12g**,

which was consistent with the observation made for the 7,7′–linked molecules.

Among the 3,2′–linked bis–indoles, replacement of a 4–BrC_6H_4 substituent **19b** by a 4–ClC_6H_4 substituent **19a** also resulted in a significant increase in β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ inhibitory activity and inhibition of *B. subtilis* growth, and a slight increase in inhibition of *E. coli* growth was also observed. Compound **19c** exhibited good activity against the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction and exclusive inhibition of *E. coli* growth.

The only 3,3′–linked bis–indole, **18**, exhibited good activity against the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction as well as inhibition of both *B. subtilis* growth and *E. coli* growth.

The only mono–indole derivative, **17**, was found to be the most active inhibitor of *B.subtilis* growth. However, the molecule showed relatively low potency in the ELISA, suggesting that it may possess a different mechanism of antibacterial activity unrelated to the inhibition of the interaction between $\sigma^{70}/\sigma^{A}{}_{2.2}$ and the β'–CH region of the core RNAP.

Considering all of the data, preliminary structure–activity relationships can be drawn for this set of compounds. Firstly, the nature of the linkage between indole units has a critical effect on the biological activity of the molecules. The 7,7′–linked compounds containing the –CO–NH–NH–CO– linker and the –CO–CO–NH–NH–CO–CO– linker inhibited both *B. subtilis* growth and *E. coli* growth. It was found that incorporation of the –CO–NH–NH–CO– linker into the 2,2′– and the 2,7′–linked molecules and the –CO–CO–NH–NH–CO– linker in to the 7,7′–, the 2,2′– and the 2,7′–linked compounds completely abolished their ability to inhibit *B. subtilis* growth. Interestingly, the 3,2′–linked molecules having the –CO–CO–NH–NH–CO– linkage were still active against *B. subtilis* growth. Furthermore, the nature of the substituent at position 3 of the indole ring could also influence the biological activity of the compounds. Changing from a 4–BrC_6H_4 substituent to a 4–ClC_6H_4 substituent enhanced biological potency across three classes of molecules: 7,7′–, 2,7′– and 3,2′–linked bis–indoles. As PhCl is smaller but more electron–withdrawing compared to PhBr, this suggests that the size and/or electronic character of this substituent may play a role in the biological activity for these types of compounds, possibly as a consequence of influencing the cellular permeability of the molecules.

Differences between inhibition of the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interaction and inhibition of bacterial growth observed for some compounds could be explained in two ways. Firstly, since many molecules were partly insoluble at *ca.* 200 μM, bacterial growth inhibitory activities need to be treated as qualitative data only. Secondly, inhibition of bacterial growth could be dependent on the ability of the novel compounds to cross the outer membrane in Gram–negative bacteria and the cell wall in Gram–positive bacteria.

Conclusion

A library of thirty novel molecules was synthesized, fully characterized and evaluated as potential inhibitors of transcription initiation complex formation. The compounds were tested for their ability to inhibit the β'–CH–$\sigma^{70}/\sigma^{A}{}_{2.2}$ interactionand for their antibacterial activity against both Gram–positive and Gram–negative bacteria in culture. Most of the molecules were found to efficiently inhibit the interaction between $\sigma^{70}/\sigma^{A}{}_{2.2}$ and the β'–CH region of the core RNAP, and showed moderate inhibition of *E. coli* growth. However, the majority of the compounds exhibited no antibacterial activity against *B.subtilis*. Structure–activity relationship studies suggested that aspects of molecular size and electronic nature may result in differences in cellular permeability, thereby influencing antibacterial activity. This research also furnished molecules capable of inhibiting the growth of Gram–negative bacteria, which is significant as discovering new compounds active against Gram–negative bacteria is much more challenging than for Gram–positive bacteria.[9] Moreover, antibiotic–resistant infections related to Gram–negative bacteria such as *Enterobacteriaceae* sp., *Salmonella* sp., *Klebsiella* sp., *Shigella* sp., *Pseudomonas* sp. and *Acinetobacter* sp. are currently a major cause of concern within the clinical environment.[3,4,7,9,31] Therefore, the identification of molecules such as **10f**, **12g**, **15c** and **19c** showing potent activity against *E. coli* (Gram–negative) growth while having no effect against *B. subtilis* (Gram–positive) growth is a significant outcome in terms of both identifying novel drug compounds as well as for combating bacterial resistance. Future research will focus on the identification and synthesis of smaller, lower molecular weight inhibitors of bacterial transcription initiation complex formation in order to overcome solubility problems and the limited permeability of the molecules through the outer membrane of the cell wall in Gram–negative bacteria.

Materials and methods

General chemistry details

Trichloroacetyl chloride was synthesized from trichloroacetic acid according to a slightly modified literature procedure.[32] All commercially available reagents and solvents were purchased from Sigma–Aldrich and Alfa Aesar. No further purification was performed for commercial chemicals. Anhydrous acetonitrile and anhydrous diethyl ether were obtained from a PureSolv MD Solvent Purification System available in the School of Chemistry at UNSW.

^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO–d_6 on a Bruker Avance III 300 MHz spectrometer in the Nuclear Magnetic Resonance Facility in the Mark Wainwright Analytical Centre at UNSW and were internally calibrated to the solvent peaks. Chemical shifts (δ) were reported in parts per million (ppm). Splitting patterns were reported as singlet (s), broad singlet (bs), doublet (d) and multiplet (m), and the observed coupling constants (J) provided in Hertz (Hz).

Infrared spectra were acquired on a Thermo Nicolet Avatar 370 FT–IR spectrophotometer using KBr discs or on a Perkin Elmer 298 IR spectrometer using paraffin mulls. The wave numbers (ν_{max}) related to the transmittance minima were reported in cm^{-1}. Ultraviolet–visible light spectra were recorded using a Varian Cary 100 Bio UV–visible spectrophotometer or a Hitachi U–3200 spectrometer. The absorption maxima (λ_{max}) in nm together with the molar absorptivities (ε) were reported. Absolute methanol and HPLC quality tetrahydrofuran were used as the solvents.

High resolution mass spectra were acquired using a Thermo Scientific LTQ Orbitrap XL LC–MS mass spectrometer (electrospray ionization mode) in the Bioanalytical Mass Spectrometry Facility in the Mark Wainwright Analytical Centre at UNSW. Masses found for hydrogen adducts ($[M+H]^+$) and sodium adducts ($[M+Na]^+$) were reported with accuracy to four decimal places. Required masses were calculated using Xcalibur software installed on the spectrometer. The EI mass spectra were recorded on a VG Quattro mass spectrometer at 70 eV ionization voltage and 200 °C ion source temperature.

Melting points were measured using a Mel–Temp melting point apparatus and are uncorrected. Temperature quoted as 0 °C was achieved with a cooling bath of ice–water.

Both gravity column chromatography and flash column chromatography were performed using Grace Davisil LC60A 40–63 micron silica gel. Reaction progress was monitored by thin–layer chromatography using Merck TLC Silica gel 60 F_{254} aluminium sheets and detection by short and long wavelength ultraviolet light.

General procedure for the synthesis of 7–trichloroacetylindoles and 2–trichloro–acetylindoles

To a solution of 3–(4–chlorophenyl)–4,6–dimethoxy–1*H*–indole **1b** (2.93 g, 10.2 mmol) in anhydrous 1,2–dichloroethane (50 mL) was added trichloroacetyl chloride (3.5 mL, 31 mmol) dropwise and the reaction mixture was heated at reflux for 3.5 h. After cooling to room temperature the reaction mixture was washed with water (1 x 50 mL), dried over anhydrous sodium sulfate, mixed with silica gel and the solvent was removed *in vacuo*. Gravity column chromatography (30 cm x 3.5 cm, dichloromethane/n–hexane, 10%/90% (v/v) → 35%/65% (v/v)) afforded the respective trichloroacetylindole derivatives in 37 and 23% yield.

3–(4–Chlorophenyl)–4,6–dimethoxy–7–trichloroacetyl–1*H*–indole (2b). Yellow solid; mp 178 °C (dichloromethane/n–hexane); UV–vis: λ_{max}(MeOH)/nm 212 (ε/$dm^3mol^{-1}cm^{-1}$ 14000), 256 (12600), 343 (8000); IR (KBr): ν_{max}/cm^{-1} 3380 (NH), 1610 (C=O), 1580, 1560, 1340, 1245, 1215, 1080;^{1}H NMR (300 MHz, $CDCl_3$): δ 3.93 (s, 3H, OMe), 4.00 (s, 3H, OMe), 6.23 (s, 1H, H5), 7.08 (d, J = 2.4 Hz, H2), 7.30–7.37 (m, 2H, ArH), 7.43–7.51 (m, 2H, ArH), 10.29 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6, 55.8 (OMe), 87.8 (C7), 98.6 (CCl_3), 98.8 (C5), 110.9 (C3), 118.7 (ArC), 121.7 (C2), 127.9, 130.9 (ArCH), 132.2, 133.9, 139.9, 160.5, 161.5 (ArC), 182.5 (C=O); MS (EI): *m/z* 435 (M+2, $Cl^{37/37}$, 7%), 433 (M, $Cl^{35/35}$, 15), 316 (33), 314 (100).

3–(4–Chlorophenyl)–4,6–dimethoxy–2–trichloroacetyl–1*H*–indole (3b). Yellow solid; mp 214 °C (dichloromethane/n–hexane); UV–vis: λ_{max}(MeOH)/nm 210 (ε/$dm^3mol^{-1}cm^{-1}$ 21200), 281 (14300), 360 (9600); IR (KBr): ν_{max}/cm^{-1} 3400 (NH), 1670 (C=O), 1615, 1570, 1380, 1350, 1250, 1210, 1150; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.63 (s, 3H, OMe), 3.88 (s, 3H, OMe), 6.13 (d, J = 1.7 Hz, 1H, H5), 6.45 (d, J = 1.7 Hz, 1H, H7), 7.36 (s, 4H, ArH), 8.96 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.4, 55.8 (OMe), 85.7 (C7), 94.1 (C5), 96.3 (CCl_3), 113.3 (C3), 120.8 (C2), 127.5, 131.8 (ArCH), 132.5, 133.0, 133.4, 139.3, 156.7, 162.6 (ArC), 170.8 (C=O); MS (EI): *m/z* 435 (M+2, $Cl^{37/37}$, 10%), 433 (M, $Cl^{35/35}$, 25), 314 (35), 279 (90), 264 (50), 150 (100).

General procedure for the synthesis of indole–7–carbohydrazides and indole–2–carbohydrazides

To a suspension of 3–(4–chlorophenyl)–4,6–dimethoxy–7–trichloroacetyl–1*H*–indole **2b** (0.94 g, 2.2 mmol) in anhydrous acetonitrile (25 mL) or 3–(4–chlorophenyl)–4,6–dimethoxy–2–trichloroacetyl–1*H*–indole **3b** (1.76 g, 4.1 mmol) in anhydrous acetonitrile (45 mL) was added hydrazine hydrate (excess, 1 mL, 20.5 mmol) and the reaction mixture was stirred at room temperature for 3.5 h. The resulting precipitate was filtered, washed with acetonitrile (3 x 10 mL), water (1 x 10 mL) and air–dried to yield the respective hydrazide in 74 and 81% yield.

3–(4–Chlorophenyl)–4,6–dimethoxy–1*H*–indole–7–carbohydrazide (4b). White solid ; mp 210–212 °C (water/acetonitrile); UV–vis: λ_{max}(THF)/nm 212 (ε/$dm^3mol^{-1}cm^{-1}$ 18500), 238 (27500), 302 (13300); IR (KBr): ν_{max}/cm^{-1} 3370 (NH), 3345 (NH), 3012, 2939, 2841, 1622 (C=O), 1591 (C=O), 1535, 1463, 1346, 1264, 1215, 1151, 1094, 1012, 983, 950, 895, 841, 796; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.88 (s, 3H, OMe), 4.02 (s, 3H, OMe), 4.59 (bs, 2H, NH_2), 6.48 (s, 1H, H5), 7.24 (d, J = 2.5 Hz, 1H, H2), 7.34–7.41 (m, 2H, ArH), 7.48–7.57 (m, 2H, ArH), 9.16 (s, 1H, NH–NH_2), 11.44 (d, J = 1.6 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.3, 56.8 (OMe), 88.1 (C7), 96.6 (C5), 110.0 (C3), 115.2 (ArC), 123.4 (C2), 127.5 (ArCH), 130.0 (ArC), 130.6 (ArCH), 134.8, 138.1, 156.0, 156.4 (ArC), 165.8 (C=O); HRMS (+ESI): found *m/z* 368.0771 ($[M+Na]^+$), $[C_{17}H_{16}ClN_3O_3Na]^+$ requires *m/z* 368.0772 (monoisotopic mass).

3–(4–Chlorophenyl)–4,6–dimethoxy–1*H*–indole–2–carbohydrazide (5b). White solid; mp 238–240 °C (water/acetonitrile); UV–vis: λ_{max}(THF)/nm 212 (ε/$dm^3mol^{-1}cm^{-1}$33200), 251 (28500), 306 (16100); IR (KBr): ν_{max}/cm^{-1} 3418 (NH), 3313 (NH), 3016, 2936, 2841, 1576 (C=O), 1503, 1462, 1278, 1202, 1139, 1091, 1041, 997, 925, 836, 814; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.59 (s, 3H, OMe), 3.77 (s, 3H, OMe), 4.35 (bs, 2H, NH_2), 6.15 (d, J = 2.0 Hz, 1H, H5), 6.50 (d, J = 2.0 Hz, 1H,

H7), 7.33–7.41 (m, 4H, ArH), 8.25 (s, 1H, NH–NH_2), 11.49 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.0, 55.3 (OMe), 86.7 (C7), 92.4 (C5), 111.2 (C3), 116.5 (ArC), 125.1 (C2), 127.0 (ArCH), 131.0 (ArC), 132.7 (ArCH), 133.9, 137.2, 154.9, 158.2 (ArC), 161.6 (C=O); HRMS (+ESI): found *m/z* 368.0779 ([M+Na]$^+$), $[C_{17}H_{16}ClN_3O_3Na]^+$ requires *m/z* 368.0772 (monoisotopic mass).

Trichloroacetyl and hydrazide derivatives of indoles with other aryl or methyl substituents at position 3 of the indole ring were synthesized following the above procedures. However, the reaction of 4,6–dimethoxy–3–methyl–1*H*–indole with trichloroacetyl chloride did not produce the corresponding 7–trichloroacetyl derivative. Gravity column chromatography afforded only 4,6–dimethoxy–3–methyl–2,7–trichloroacetyl–1*H*–indole as the faster moving batch (R_f = 0.54) and 4,6–dimethoxy–3–methyl–2–trichloroacetyl–1*H*–indole as the slower moving batch (R_f = 0.42).

General procedure for the synthesis of indol–2–yl–glyoxyloyl chlorides and indol–7–yl–glyoxyloyl chlorides

The synthesis followed an established general procedure.[28] To a solution of 3–(4–chlorophenyl)–4,6–dimethoxy–1*H*–indole **1b** (1.75 g, 6.08 mmol) in anhydrous diethyl ether (35 mL) at 0 °C was added with oxalyl chloride (1.6 mL, 18 mmol) and the reaction mixture was warmed from 0 °C to room temperature, and stirred for 3 h and resulted in 33, 43 and 92% yield.

3–(4–Chlorophenyl)–4,6–dimethoxy–1*H*–indol–7–yl–glyoxyloyl chloride (6b). The filtrate was mixed with silica gel, solidified *in vacuo* and purified by gravity column chromatography (dichloromethane/n–hexane, 80%/20% (v/v)) to afford *the title product* **6b** as a yellow–orange solid *lit,*[28]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.95 (s, 3H, OMe), 4.00 (s, 3H, OMe), 6.19 (s, 1H, H5), 7.10 (d, *J* = 2.3 Hz, 1H, H2), 7.31–7.38 (m, 2H, ArH), 7.43–7.50 (m, 2H, ArH), 10.36 (bs, 1H, NH); HRMS (+ESI): found *m/*z 378.0311 ([M+H]$^+$), $[C_{18}H_{13}Cl_2NO_4H]^+$ requires 378.0294 (monoisotopic mass).

3–(4–Chlorophenyl)–4,6–dimethoxy–1*H*–indol–2–yl–glyoxyloyl chloride (7b). The resulting precipitate was filtered out from the reaction mixture, washed with anhydrous diethyl ether (1 x 30 mL) and dried to yield *the title product* **7b** as a dark red solid *lit*,[28]; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.58 (s, 3H, OMe), 3.80 (s, 3H, OMe), 6.15 (d, *J* = 1.9 Hz, 1H, H5), 6.52 (d, *J* = 1.9 Hz, 1H, H7), 7.29–7.39 (m, 4H, ArH), 11.97 (s, 1H, NH); HRMS (+ESI): found *m/z* 378.0311 ([M+H]$^+$), $[C_{18}H_{13}Cl_2NO_4H]^+$ requires 378.0294 (monoisotopic mass).

Glyoxyloyl chlorides of indoles with other aryl or methyl substituents at position 3 of the indole ring were synthesized following the above procedures. However, only trace amounts (<0.5%) of the 7–glyoxyloyl chloride were produced in reaction of 4,6–dimethoxy–3–methyl–1*H*–indole and oxalyl chloride. Additional recrystallization from hot ethyl acetate was also required to purify the corresponding 2–glyoxyloyl chloride.

1*H*–Indol–3–yl–glyoxyloyl chloride (9). To a solution of indole **8** (2.67 g, 22.8 mmol) in anhydrous diethyl ether (50 mL) at 0 °C was added oxalyl chloride (6.1 mL, 69 mmol) in one go and the reaction mixture was warmed from 0 °C to room temperature, and stirred for further 3 h. The resulting precipitate was filtered out from the reaction mixture, washed with anhydrous diethyl ether and dried to yield *the title product* **9** as a dark red solid *lit,*[29]; ^{1}H NMR (300 MHz, DMSO–d_6): δ 7.23–7.31 (m, 2H, H5, H6), 7.52–7.58 (m, 1H, H7), 8.16–8.21 (m, 1H, H4), 8.43 (d, *J* = 3.3 Hz, 1H, H2), 12.37 (s, 1H, NH).

General procedure for the synthesis of 10a–f, 11a–c, 12a–g, 13, 14a–c, 15a–d and 19a–c

To a solution or suspension of the appropriate 2–trichloroacetylindole **3a–f**, 7–trichloroacetylindole **2a–e**, 2–glyoxyloyl chloride **7a–d**, 7–glyoxyloyl chloride **6a–c** or 3–glyoxyloyl chloride **9** (1.0 equiv) in acetonitrile was added the appropriate indole–7–carbohydrazide **4a–e** or indole–2–carbohydrazide **5a–f** (1.0 equiv) followed by triethylamine (5 drops) and the reaction mixture was stirred at room temperature (reactions involving glyoxyloyl chlorides or trichloroacetylindole derivatives) or at reflux (reactions involving trichloroacetylindole derivatives) until the TLC showed the completion of the reaction (5–26 h). The solvent was evaporated and water was added to the residue. The resulting precipitate was filtered, dried and recrystallized from methanol giving the product as a pale brown, grey, yellow or orange solid in the range of 40–65% yield.

3–(4–Chlorophenyl)–*N′*–(3–(4–chlorophenyl)–4,6–dimethoxy–1*H*–indole–7–carbonyl)–4,6–dimethoxy–1*H*–indole–7–carbohydrazide (10a). Brown solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 243 (ε/dm^{3}mol^{-1}cm^{-1} 86600), 279 (45800), 348 (57200), 364 (42600); IR (KBr): ν_{max}/cm^{-1} 3388 (NH), 1592 (C=O), 1448, 1340, 1213, 1151, 792; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.93 (s, 6H, OMe), 4.13 (s, 6H, OMe), 6.58 (s, 2H, H5), 7.26 (d, *J* = 2.5 Hz, 2H, H2), 7.34–7.43 (m, 4H, ArH), 7.49–7.59 (m, 4H, ArH), 10.52 (s, 2H, NH), 11.52 (d, *J* = 2.0 Hz, 2H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.5, 57.2 (OMe), 88.3 (C5), 95.8 (C7), 110.2 (C3), 115.4 (ArC), 123.6 (C2), 127.5 (ArCH), 130.1 (ArC), 130.7 (ArCH), 134.6, 138.0, 156.6, 157.2 (ArC), 163.1 (C=O); HRMS (+ESI): found *m/z* 681.1273 ([M+Na]$^+$), $[C_{34}H_{28}Cl_2N_4O_6Na]^+$ requires *m/z* 681.1278 (monoisotopic mass).

3–(4–Bromophenyl)–*N′*–(3–(4–bromophenyl)–4,6–dimethoxy–1*H*–indole–7–carbonyl)–4,6–dimethoxy–1*H*–indole–7–carbohydrazide (10b). Pale brown solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 242 (ε/dm^{3}mol^{-1} cm^{-1} 65200), 281 (34400), 348 (42600), 365 (31600); IR (KBr): ν_{max}/cm^{-1} 3377 (NH), 1614 (C=O), 1593 (C=O), 1444, 1335, 1218, 1150, 1109, 979, 795; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.93 (s, 6H, OMe), 4.13 (s, 6H, OMe), 6.58 (s, 2H, H5), 7.26 (d, *J* = 2.4 Hz, 2H, H2), 7.42–7.59 (m, 8H, ArH), 10.51 (s, 2H, NH), 11.53 (d, *J* = 1.6 Hz, 2H, NH); HRMS (+ESI): found *m/z*

747.0440 ([M+H]$^+$), [$C_{34}H_{28}Br_2N_4O_6H$]$^+$ requires *m/z* 747.0448 (monoisotopic mass). The compound was not soluble enough in DMSO–$d_6$for ^{13}C NMR measurement.

4,6–Dimethoxy–*N′*–(4,6–dimethoxy–3–(4–fluorophenyl)–1*H*–indole–7–carbonyl)–3–(4–fluorophenyl)–1*H*–indole–7–carbohydrazide (10c). Light brown solid; mp > 300 ºC (methanol); IR (KBr): ν_{max}/cm^{-1} 3370 (NH), 1616 (C=O), 1592 (C=O), 1444, 1339, 1215, 1150, 1105, 979, 793; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.79 (s, 6H, OMe), 3.94 (s, 6H, OMe), 6.41 (s, 2H, H5), 7.32 (d, *J* = 2.2 Hz, 2H, H2), 7.29–7.49 (m, 8H, ArH), 10.58 (s, 2H, NH), 11.48 (bs, 2H, NH); HRMS (+ESI): found *m/z* 627.2055 ([M+H]$^+$), [$C_{34}H_{28}F_2N_4O_6H$]$^+$ requires *m/z* 627.2050 (monoisotopic mass). The compound was not soluble enough in DMSO–d_6 for ^{13}C NMR and in THF for UV–vis measurements.

4,6–Dimethoxy–*N′*–(4,6–dimethoxy–3–(4–methoxyphenyl)–1*H*–indole–7–carbonyl)–3–(4–methoxyphenyl)–1*H*–indole–7–carbohydrazide (10d). Brown solid; mp > 300 ºC (methanol); IR (KBr): ν_{max}/cm^{-1} 3376 (NH), 1620 (C=O), 1590 (C=O), 1443, 1339, 1216, 1150, 1105, 978, 790; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.79 (s, 3H, OMe), 3.85 (s, 3H, OMe), 3.91 (s, 6H, OMe), 4.03 (s, 6H, OMe), 6.47 (s, 2H, H5), 7.35 (d, *J* = 2.2 Hz, 2H, H2), 7.40–7.59 (m, 8H, ArH), 10.44 (s, 2H, NH), 11.51 (bs, 2H, NH); HRMS (+ESI): found *m/z* 651.2449 ([M+H]$^+$), [$C_{36}H_{34}N_4O_8H$]$^+$requires *m/z* 651.2449 (monoisotopic mass). The compound was not soluble enough in DMSO–d_6 for ^{13}C NMR and in THF for UV–vis measurements.

4,6–Dimethoxy–*N′*–(4,6–dimethoxy–3–(4–tolyl)–1*H*–indole–7–carbonyl)–3–(4–tolyl)–1*H*–indole–7–carbohydrazide (10e). Pale brown solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 68400), 245 (72200), 269 (46400), 348 (47200), 365 (36400); IR (KBr): ν_{max}/cm^{-1} 3366 (NH), 1615 (C=O), 1591 (C=O), 1448, 1338, 1216, 1151, 1107, 979, 790; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.31 (s, 6H, Me), 3.89 (s, 6H, OMe), 4.05 (s, 6H, OMe), 6.51 (s, 2H, H5), 7.22 (d, *J* = 2.3 Hz, 2H, H2), 7.38–7.51 (m, 8H, ArH), 10.65 (s, 2H, NH), 11.56 (bs, 2H, NH); HRMS (+ESI): found *m/z* 619.2554 ([M+H]$^+$), [$C_{36}H_{34}N_4O_6H$]$^+$ requires *m/z* 619.2551 (monoisotopic mass). The compound was not soluble enough in DMSO–d_6 for ^{13}C NMR measurement.

3–(4–Bromophenyl)–*N′*–(3–(4–chlorophenyl)–4,6–dimethoxy–1*H*–indole–7–carbonyl)–4,6–dimethoxy–1*H*–indole–7–carbohydrazide (10f). Pale yellow solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 210 (ε/dm^{3}mol^{-1}cm^{-1}65100), 239 (70400), 275 (41000), 329 (38900); IR (KBr): ν_{max}/cm^{-1} 3415 (NH), 1626 (C=O), 1580 (C=O), 1560, 1536, 1465, 1351, 1323, 1214, 1183, 1150, 1089, 980, 796; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.93 (s, 3H, OMe), 3.96 (s, 3H, OMe), 3.98 (s, 3H, OMe), 4.12 (s, 3H, OMe), 6.51 (s, 1H, H5), 6.57 (s, 1H, H5), 7.25 (d, *J* = 2.3 Hz, 1H, H2), 7.29 (d, *J* = 2.3 Hz, 1H, H2), 7.35–7.67 (m, 8H, ArH), 9.94 (s, 1H, NH), 10.89 (s, 1H, NH), 11.44 (d, *J* = 1.8 Hz, 1H, NH), 11.56 (d, *J* = 1.8 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.5, 55.9, 57.2, 57.3 (OMe), 88.4, 88.7 (C5), 96.2, 101.6 (C7), 109.9, 110.2 (C3), 115.4, 116.4,118.9 (ArC), 123.6, 123.7 (C2), 127.5 (ArCH), 130.1 (ArC), 130.5, 130.7, 131.1 (ArCH), 134.7, 137.1, 138.1, 156.6, 157.2, 161.0, 162.0 (ArC), 164.6, 165.9 (C=O); HRMS (+ESI): found *m/z* 703.0948 ([M+H]$^+$), [$C_{34}H_{28}BrClN_4O_6H$]$^+$ requires *m/z* 703.0954 (monoisotopic mass).

3–(4–Chlorophenyl)–*N′*–(3–(4–chlorophenyl)–4,6–dimethoxy–1*H*–indole–2–carbonyl)–4,6–dimethoxy–1*H*–indole–2–carbohydrazide (11a). White solid; mp 292–294 ºC (methanol); UV–vis: λ_{max}(THF)/nm 214 (ε/dm^{3}mol^{-1}cm^{-1} 64500), 254 (49600), 315 (26100); IR (KBr): ν_{max}/cm^{-1} 3354 (NH), 1626 (C=O), 1536, 1259, 1210, 1132, 1089, 811; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.60 (s, 6H, OMe), 3.83 (s, 6H, OMe), 6.12 (d, *J* = 1.7 Hz, 2H, H5), 6.41 (d, *J* = 1.7 Hz, 2H, H7), 7.45 (s, 8H, ArH), 7.77 (bs, 2H, NH), 9.13 (bs, 2H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.2, 55.8 (OMe), 86.0 (C5), 93.3 (C7), 113.0 (C3), 119.0 (ArC), 122.2 (C2), 128.8, 132.2 (ArCH), 132.9, 134.3, 137.5, 156.1, 159.1 (ArC), 160.3 (C=O); HRMS (+ESI): found *m/z* 681.1292 ([M+Na]$^+$), [$C_{34}H_{28}Cl_2N_4O_6Na$]$^+$ requires *m/z* 681.1278 (monoisotopic mass).

3–(4–Chlorophenyl)–4,6–dimethoxy–*N′*–(4,6–dimethoxy–3–methyl–1*H*–indole–2–carbonyl)–1*H*–indole–2–carbohydrazide (11b). Grey solid; mp 274–276 °C (methanol); UV–vis: λ_{max}(THF)/nm 214 (ε/dm^{3}mol^{-1}cm^{-1} 46400), 250 (40000), 313 (33600); IR (KBr): ν_{max}/cm^{-1} 3319 (NH), 2933 (NH), 2840 (NH), 1644 (C=O), 1584 (C=O), 1533, 1463, 1322, 1258, 1216, 1153, 1043, 991, 941, 820; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.63 (s, 3H, Me), 3.61 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.82 (s, 3H, OMe), 6.12 (d, *J* = 1.7 Hz, 1H, H5), 6.19 (d, *J* = 1.6 Hz, 1H, H5), 6.41 (d, *J* = 1.7 Hz, 1H, H7), 6.56 (d, *J* = 1.6 Hz, 1H, H7), 7.38 (d, *J* = 8.4 Hz, 2H, ArH), 7.46 (d, *J* = 8.4 Hz, 2H, ArH), 9.21 (s, 1H, NH), 9.70 (s, 1H, NH), 11.01 (s, 1H, NH), 11.66 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 12.0 (Me), 55.0, 55.2, 55.2, 55.3 (OMe), 86.4, 86.7 (C7), 91.7, 92.6 (C5), 111.3, 112.6 (C3), 116.6, 118.2, 123.1, 123.9 (ArC), 127.0 (ArCH), 131.2 (ArC), 132.9 (ArCH), 133.6, 137.6, 137.7, 155.1, 156.0, 158.6, 158.7 (ArC), 160.7, 161.1 (C=O); HRMS (+ESI): found *m/z* 585.1492 ([M+Na]$^+$), [$C_{29}H_{27}ClN_4O_6Na$]$^+$ requires *m/z* 585.1511 (monoisotopic mass).

4,6–Dimethoxy–*N′*–(4,6–dimethoxy–3–methyl–1*H*–indole–2–carbonyl)–3–methyl–1*H*–indole–2–carbohydrazide (11c). Light brown solid; mp 288–290 °C (methanol); UV–vis: λ_{max}(THF)/nm 215 (ε/dm^{3}mol^{-1}cm^{-1} 23300), 246 (27000), 312 (33600); IR (KBr): ν_{max}/cm^{-1} 3288 (NH), 3185, 2932 (NH), 2837 (NH), 2135, 2069, 1651 (C=O), 1423, 1380, 1098, 1060, 998, 938, 876, 806; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.67 (s, 6H, Me), 3.77 (s, 6H, OMe), 3.83 (s, 6H, OMe), 6.13 (d, *J* = 1.8 Hz, 2H, H5), 6.44 (d, *J* = 1.8 Hz, 2H, H7), 9.69 (s, 2H, NH), 11.05 (s, 2H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 12.1 (Me), 55.2 (2 x OMe), 86.5 (C7), 91.5 (C5), 112.6 (C3), 116.4 (C3a), 123.4 (C2), 137.7 (C7a), 156.0, 158.7 (ArC), 161.5 (C=O); HRMS (+ESI): found *m/z* 489.1745 ([M+Na]$^+$), [$C_{24}H_{26}N_4O_6Na$]$^+$ requires *m/z* 489.1745 (monoisotopic mass).

3–(4–Chlorophenyl)–4,6–dimethoxy–*N′*–(4,6–dimethoxy–3–(4–methoxyphenyl)–1*H*–indole–7–carbonyl)–1*H*–indole–2–carbohydrazide (12a). Grey–brown solid; mp 174–176 ºC (methanol); UV–vis: λ_{max}(THF)/nm 213 (ε/dm^{3}mol^{-1}cm^{-1} 67800), 252 (68400), 315 (36100), 339 (38100); IR (KBr):

ν_{max}/cm^{-1} 3388 (NH), 1620 (C=O), 1590 (C=O), 1539, 1455, 1344, 1244, 1213, 1152, 1138, 805; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.60 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.05 (s, 3H, OMe), 6.18 (d, J = 1.5 Hz, 1H, H5), 6.49 (s, 1H, H5), 6.56 (d, J = 1.5 Hz, 1H, H7), 6.82–7.02 (m, 2H, ArH), 7.12 (d, J = 2.3 Hz, 1H, H2), 7.27–7.62 (m, 6H, ArH), 9.36 (s, 1H, NH), 9.99 (s, 1H, NH), 11.25 (s, 1H, NH), 11.70 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.0, 55.0, 55.3, 55.4, 57.0 (OMe), 86.6, 87.9 (C5), 92.6, 95.6 (C7), 110.4, 111.5 (C3), 113.1 (ArCH), 116.4, 118.5 (ArC), 122.5, 123.5 (C2), 127.0 (ArCH), 128.1 (ArC), 130.1 (ArCH), 131.3 (ArC), 132.8 (ArCH), 133.6, 137.6, 138.0, 155.2, 156.5, 157.4, 157.4, 158.7 (ArC), 159.7, 164.5 (C=O); HRMS (+ESI): found m/z 655.1955 ([M+H]$^+$), [$C_{35}H_{31}ClN_4O_7H$]$^+$ requires m/z 655.1954 (monoisotopic mass).

3–(4–Chlorophenyl)–4,6–dimethoxy–*N′*–(4,6–dimethoxy–3–(4–tolyl)–1*H*–indole–7–carbonyl)–1*H*–indole–2–carbohydrazide (12b). Grey–brown solid; mp 176–178 ºC (methanol); UV–vis: λ_{max}(THF)/nm 213 (ε/dm^{3}mol^{-1}cm^{-1} 102700), 248 (94600), 312 (51800); IR (KBr): ν_{max}/cm^{-1} 3372 (NH), 1622 (C=O), 1593 (C=O), 1539, 1456, 1342, 1248, 1213, 1152, 1137, 808; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.32 (s, 3H, Me), 3.60 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.89 (s, 3H, OMe), 4.05 (s, 3H, OMe), 6.18 (d, J = 1.6 Hz, 1H, H5), 6.50 (s, 1H, H5), 6.56 (d, J = 1.6 Hz, 1H, H7), 7.13 (s, 1H, H2), 7.13–7.56 (m, 8H, ArH), 9.35 (d, J = 2.1 Hz, 1H, NH), 9.99 (d, J = 1.8 Hz, 1H, NH), 11.27 (d, J = 1.7 Hz, 1H, NH), 11.68 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 21.9 (Me), 55.4, 55.8, 55.8, 57.4 (OMe), 87.1, 88.3 (C5), 93.2, 96.1 (C7), 110.9, 112.0 (C3), 116.9 (ArC), 122.9 (C2), 127.6, 128.5 (ArCH), 128.7 (ArC), 130.6 (ArCH), 131.8, 132.3 (ArC), 133.3 (ArCH), 134.1, 136.3, 136.9, 138.0, 138.4, 157.0, 157.8, 159.2, 159.9 (ArC), 160.2, 165.1 (C=O); HRMS (+ESI): found m/z 661.1847 ([M+Na]$^+$), [$C_{35}H_{31}ClN_4O_6Na$]$^+$ requires m/z 661.1824 (monoisotopic mass).

4,6–Dimethoxy–*N′*–(4,6–dimethoxy–3–(4–tolyl)–1*H*–indole–2–carbonyl)–3–(4–tolyl)–1*H*–indole–7–carbohydrazide (12c). Grey solid; mp 170–172 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1}52800), 250 (52200), 313 (26800), 332 (26600); IR (KBr): ν_{max}/cm^{-1} 3409 (NH), 1616 (C=O), 1589 (C=O), 1542, 1460, 1344, 1247, 1213, 1151, 1137, 807; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.32 (s, 3H, Me), 2.37 (s, 3H, Me), 3.57 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.04 (s, 3H, OMe), 6.15 (d, J = 1.5 Hz, 1H, H5), 6.48 (s, 1H, H5), 6.56 (d, J = 1.5 Hz, 1H, H7), 7.13 (s, 1H, H2), 7.13–7.49 (m, 8H, ArH), 8.57 (d, J = 2.4 Hz, 1H, NH), 9.98 (d, J = 2.0 Hz, 1H, NH), 11.28 (s, 1H, NH), 11.64 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 20.7, 21.0 (Me), 55.0, 55.2, 55.4, 56.9 (OMe), 86.6, 87.9 (C5), 92.5, 95.5 (C7), 110.3, 111.9 (C3),116.6, 119.3 (ArC), 122.8, 123.2 (C2), 128.2, 128.3, 129.0, 130.7 (ArCH), 131.7, 132.8, 134.3, 136.1, 137.7, 138.0, 155.4, 156.6, 157.4, 158.7 (ArC), 160.0, 164.5 (C=O); HRMS (+ESI): found m/z 619.2550 ([M+H]$^+$), [$C_{36}H_{34}N_4O_6H$]$^+$ requires m/z 619.2551 (monoisotopic mass).

4,6–Dimethoxy–*N′*–(4,6–dimethoxy–3–(4–fluorophenyl)–1*H*–indole–7–carbonyl)–3–(4–tolyl)–1*H*–indole–2–carbohydrazide (12d). Grey solid; mp 168–170 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 62200), 249 (57800), 313 (28500), 336 (30700); IR (KBr): ν_{max}/cm^{-1} 3390 (NH), 1615 (C=O), 1594 (C=O), 1542, 1461, 1344, 1256, 1213, 1151, 1136, 804; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.39 (s, 3H, Me), 3.58 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.92 (s, 3H, OMe), 4.06 (s, 3H, OMe), 6.17 (d, J = 1.5 Hz, 1H, H5), 6.52 (s, 1H, H5), 6.58 (d, J = 1.5 Hz, 1H, H7), 7.23 (d, J = 2.7 Hz, 1H, H2), 7.25–7.52 (m, 8H, ArH), 8.58 (d, J = 2.7 Hz, 1H, NH), 10.00 (d, J = 2.7 Hz, 1H, NH), 11.40 (s, 1H, NH), 11.65 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 21.4 (Me), 55.5, 55.7, 55.8, 57.4 (OMe), 87.0, 88.4 (C5), 93.0, 96.0 (C7), 110.8, 112.4 (C3), 116.6 (ArCH), 117.1 (ArC), 123.3 (C2), 128.7, 129.4, 131.2 (ArCH), 132.1, 133.3, 134.8, 136.1, 136.6, 138.1, 138.4, 155.8, 157.1, 157.9, 159.2, 162.0 (ArC), 160.4, 164.9 (C=O); HRMS (+ESI): found m/z 623.2301 ([M+H]$^+$), [$C_{35}H_{31}FN_4O_6H$]$^+$ requires m/z 623.2300 (monoisotopic mass).

***N′*–(3–(4–Bromophenyl)–4,6–dimethoxy–1*H*–indole–7–carbonyl)–4,6–dimethoxy–3–(4–tolyl)–1*H*–indole–2–carbohydrazide (12e).** Creamy white solid; mp 238–240 ºC (methanol); UV–vis: λ_{max}(THF)/nm 213 (ε/dm^{3}mol^{-1}cm^{-1} 113400), 248 (117600), 309 (67500), 332 (68700); IR (KBr): ν_{max}/cm^{-1} 3377 (NH), 1622 (C=O), 1586 (C=O), 1540, 1461, 1346, 1257, 1213, 1152, 1139, 805; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.37 (s, 3H, Me), 3.56 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.90 (s, 3H, OMe), 4.04 (s, 3H, OMe), 6.15 (d, J = 1.7 Hz, 1H, H5), 6.50 (s, 1H, H5), 6.55 (d, J = 1.7 Hz, 1H, H7), 7.06–7.60 (m, 9H, H2, ArH), 8.56 (d, J = 2.8 Hz, 1H, NH), 9.98 (d, J = 2.3 Hz, 1H, NH), 11.39 (s, 1H, NH), 11.63 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 20.9 (Me), 55.0, 55.2, 55.5, 57.0 (OMe), 86.6, 88.1 (C5), 92.5, 95.7 (C7), 110.0, 111.9 (C3), 115.4, 118.5 (ArC), 123.2, 123.5 (C2), 128.3, 130.4 (ArCH), 130.6 (ArC), 130.7,131.0 (ArCH), 131.7, 135.0, 136.1, 137.7, 138.0, 155.4, 156.7, 157.3, 158.7 (ArC), 160.0, 164.4 (C=O); HRMS (+ESI): found m/z 683.1518 ([M+H]$^+$), [$C_{35}H_{31}BrN_4O_6H$]$^+$ requires m/z 683.1500 (monoisotopic mass).

***N′*–(3–(4–Bromophenyl)–4,6–dimethoxy–1*H*–indole–7–carbonyl)–4,6–dimethoxy–3–methyl–1*H*–indole–2–carbohydrazide (12f).** Grey solid; mp 280–282 °C (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 38200), 243 (43600), 334 (33100); IR (KBr): ν_{max}/cm^{-1} 3442 (NH), 3406 (NH), 2936 (NH), 2838 (NH), 1590 (C=O), 1534, 1464, 1344, 1212, 1154, 1010, 981, 809, 794; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.71 (s, 3H, Me), 3.78 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.92 (s, 3H, OMe), 4.10 (s, 3H, OMe), 6.14 (d, J = 1.8 Hz, 1H, H5), 6.45 (d, J = 1.8 Hz, 1H, H7), 6.55 (s, 1H, H5), 7.26 (d, J = 2.5 Hz, 1H, H2), 7.43–7.57 (m, 4H, ArH), 9.99 (d, J = 1.9 Hz, 1H, NH), 10.11 (d, J = 2.4 Hz, 1H, NH), 11.13 (s, 1H, NH), 11.49 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 12.1 (Me), 55.2, 55.2, 55.5, 57.1 (OMe), 86.5 (C7), 88.3, 91.8 (C5), 96.2 (C7), 110.1, 112.6 (C3), 115.4, 116.4, 118.6, 123.3 (ArC), 123.6 (C2), 130.4, 131.1 (ArCH), 135.1, 137.7, 138.2, 156.0, 156.7, 157.2, 158.7 (ArC), 160.8, 164.9 (C=O); HRMS (+ESI): found m/z 629.0984 ([M+Na]$^+$), [$C_{29}H_{27}BrN_4O_6Na$]$^+$ requires m/z 629.1006 (monoisotopic mass).

***N′*–(3–(4–Chlorophenyl)–4,6–dimethoxy–1*H*–indole–7–carbonyl)–4,6–dimethoxy–3–methyl–1*H*–indole–2–carbohydrazide (12g).** Grey solid; mp 282–284 °C (methanol); UV–vis: λ_{max}(THF)/nm 243 (ε/dm^{3}mol^{-1}cm^{-1} 43800), 334 (36400); IR (KBr): ν_{max}/cm^{-1} 3449 (NH), 3405 (NH), 2937 (NH), 2839 (NH), 1595 (C=O), 1537, 1464, 1345, 1214, 1154, 1137, 982, 806, 794; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.71 (s, 3H, Me), 3.78 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.92 (s, 3H, OMe), 4.10 (s, 3H, OMe), 6.14 (d, J = 1.7 Hz, 1H, H5), 6.45 (d, J = 1.7 Hz, 1H, H7), 6.55 (s, 1H, H5), 7.26 (d, J = 2.4 Hz, 1H, H2), 7.38 (d, J = 8.6 Hz, 2H, ArH), 7.54 (d, J = 8.6 Hz, 2H, ArH), 10.00 (d, J = 2.0 Hz, 1H, NH), 10.11 (d, J = 2.6 Hz, 1H, NH), 11.13 (s, 1H, NH), 11.49 (d, J = 1.5 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 12.1 (Me), 55.2, 55.2, 55.5, 57.0 (OMe), 86.5 (C7), 88.3, 91.7 (C5), 96.2 (C7), 110.2, 112.6 (C3), 115.4, 116.4, 123.3 (ArC), 123.5 (C2), 127.5 (ArCH), 130.1 (ArC), 130.7 (ArCH), 134.7, 137.7, 138.2, 156.0, 156.7, 157.2, 158.7 (ArC), 160.8, 164.9 (C=O); HRMS (+ESI): found *m/z* 585.1514 ([M+Na]$^+$), [$C_{29}H_{27}ClN_4O_6Na$]$^+$ requires *m/z* 585.1511 (monoisotopic mass).

***N′*–(2–(3–(4–Bromophenyl)–4,6–dimethoxy–1*H*–indol–2–yl)–2–oxoacetyl)–4,6–dimethoxy–3–methyl–1*H*–indole–2–carbohydrazide (13).** Orange solid; mp 286–288 °C (methanol); UV–vis: λ_{max}(THF)/nm 214 (ε/dm^{3}mol^{-1}cm^{-1} 97100), 246 (58600), 307 (42500); IR (KBr): ν_{max}/cm^{-1} 3405 (NH), 2936 (NH), 2839 (NH), 1620 (C=O), 1522, 1463, 1383, 1318, 1267, 1210, 1155, 1133, 812; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.65 (s, 3H, Me), 3.60 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.83 (s, 3H, OMe), 6.13 (d, J = 1.9 Hz, 1H, H5), 6.17 (d, J = 1.9 Hz, 1H, H5), 6.42 (d, J = 1.9 Hz, 1H, H7), 6.68 (d, J = 1.9 Hz, 1H, H7), 7.29–7.37 (m, 2H, ArH), 7.45–7.52 (m, 2H, ArH), 9.58 (s, 1H, NH), 10.90 (bs, 1H, NH), 11.08 (s, 1H, NH), 11.89 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 12.0 (Me), 55.2 (3 x OMe), 55.4 (OMe), 86.5, 86.7 (C7), 91.8, 93.5 (C5), 112.2, 112.6 (C3), 116.6, 120.4, 123.0, 126.4, 126.7 (ArC), 129.6, 132.8 (ArCH), 133.5, 137.8, 139.9, 156.0, 156.0, 158.8, 160.6 (ArC), 160.9, 162.9, 178.1 (C=O); HRMS (+ESI): found *m/z* 657.0940 ([M+Na]$^+$), [$C_{30}H_{27}BrN_4O_7Na$]$^+$ requires *m/z* 657.0955 (monoisotopic mass).

***N′*–(2–(3–(4–Chlorophenyl)–4,6–dimethoxy–1*H*–indol–2–yl)–2–oxoacetyl)–4,6–dimethoxy–3–(4–methoxyphenyl)–1*H*–indole–7–carbohydrazide (14a).** Orange solid; mp 288–290 ºC (methanol); UV–vis: λ_{max}(THF)/nm 214 (ε/dm^{3}mol^{-1}cm^{-1} 62100), 245 (50400), 263 (42700), 343 (29800); IR (KBr): ν_{max}/cm^{-1} 3362 (NH), 1617 (C=O), 1595 (C=O), 1541, 1451, 1350, 1259, 1211, 1152, 1137, 805; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.57 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.93 (s, 3H, OMe), 3.96 (s, 3H, OMe), 4.05 (s, 3H, OMe), 6.13 (d, J = 1.5 Hz, 1H, H5), 6.36 (d, J = 1.5 Hz, 1H, H7), 6.58 (s, 1H, H5), 7.07–7.49 (m, 9H, H2, ArH), 9.57 (s, 1H, NH), 11.33 (d, J = 1.5 Hz, 1H, NH), 11.82 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.6, 55.6, 55.8, 56.0, 57.7 (OMe), 87.0, 88.7 (C5), 93.6, 96.0 (C7), 110.9, 112.9 (C3), 114.6 (ArCH), 116.3 (ArC), 123.7 (C2), 127.4 (ArC), 127.5 (ArCH), 128.9 (ArC), 131.2, 131.3 (ArCH), 132.5, 132.6, 136.5, 138.4, 140.4, 156.8, 157.1, 157.9, 159.6, 159.8 (ArC), 163.1, 164.4, 180.2 (C=O); HRMS (+ESI): found *m/z* 683.1900 ([M+H]$^+$), [$C_{36}H_{31}ClN_4O_8H$]$^+$ requires *m/z* 683.1903 (monoisotopic mass).

4,6–Dimethoxy–3–(4–fluorophenyl)–*N′*–(2–(4,6–dimethoxy–3–(4–tolyl)–1*H*–indol–2–yl)–2–oxoacetyl)–1*H*–indole–7–carbohydrazide (14b). Yellow solid; mp 282–284 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 60000), 242 (48200), 343 (24500); IR (KBr): ν_{max}/cm^{-1} 3380 (NH), 1615 (C=O), 1578 (C=O), 1544, 1455, 1352, 1257, 1211, 1159, 1138, 806; ^{1}H NMR (300 MHz, DMSO–d_6): δ 2.27 (s, 3H, Me), 3.57 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.90 (s, 3H, OMe), 4.06 (s, 3H, OMe), 6.15 (d, J = 1.8 Hz, 1H, H5), 6.53 (s, 1H, H5), 6.62 (d, J = 1.8 Hz, 1H, H7), 7.00–7.36 (m, 7H, H2, ArH), 7.44–7.62 (m, 2H, ArH), 9.42 (s, 1H, NH), 11.06 (bs, 1H, NH),11.36 (d, J = 1.5 Hz, 1H, NH), 11.84 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 20.9 (Me), 55.2, 55.4, 55.5, 57.3 (OMe), 86.5, 88.3 (C5), 93.2, 95.6 (C7), 110.5, 112.5 (C3), 114.2, 114.5 (ArCH), 115.7 (ArC), 123.4 (C2), 127.1 (ArCH), 127.5, 128.1 (ArC), 130.9 (ArCH), 132.1, 132.1, 136.0, 137.9, 140.0, 156.4, 156.7, 157.5, 160.9 (ArC), 162.6, 164.0, 179.8 (C=O); HRMS (+ESI): found *m/z* 651.2255 ([M+H]$^+$), [$C_{36}H_{31}FN_4O_7H$]$^+$ requires *m/z* 651.2250 (monoisotopic mass).

***N′*–(2–(3–(4–Chlorophenyl)–4,6–dimethoxy–1*H*–indol–2–yl)–2–oxoacetyl)–4,6–dimethoxy–3–(4–fluorophenyl)–1*H*–indole–7–carbohydrazide (14c).** Yellow–orange solid; mp 270–272 ºC (methanol); UV–vis: λ_{max}(THF)/nm 214 (ε/dm^{3}mol^{-1}cm^{-1} 66200), 243 (60600), 263 (48300), 342 (34600); IR (KBr): ν_{max}/cm^{-1} 3381 (NH), 1615 (C=O), 1578 (C=O), 1544, 1454, 1350, 1257, 1218, 1158, 1135, 805; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.59 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.91 (s, 3H, OMe), 4.09 (s, 3H, OMe), 6.17 (d, J = 1.7 Hz, 1H, H5), 6.53 (s, 1H, H5), 6.63 (d, J = 1.7 Hz, 1H, H7), 7.08–7.60 (m, 9H, H2, ArH), 9.52 (s, 1H, NH), 11.14 (bs, 1H, NH), 11.37 (d, J = 1.5 Hz, 1H, NH), 11.95 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.3, 55.5, 55.5, 57.2 (OMe), 86.6, 88.2 (C5), 93.4, 95.4 (C7), 110.4, 112.4 (C3), 114.2, 114.5 (ArCH), 115.7 (ArC), 123.3 (C2), 126.3 (ArC), 126.6 (ArCH), 127.4 (ArC), 130.8, 130.9 (ArCH), 131.9, 132.1 (ArC), 132.7 (ArCH), 132.9, 138.0, 139.9, 156.2, 156.8, 157.5, 161.0 (ArC), 162.5, 164.1, 179.6 (C=O); HRMS (+ESI): found *m/z* 671.1712 ([M+H]$^+$), [$C_{35}H_{28}ClFN_4O_7H$]$^+$ requires *m/z* 671.1703 (monoisotopic mass).

3–(4–Bromophenyl)–*N′*–(2–(3–(4–bromophenyl)–4,6–dimethoxy–1*H*–indol–7–yl)–2–oxoacetyl)–4,6–dimethoxy–1*H*–indole–7–carbohydrazide (15a). Pale yellow solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 211(ε/dm^{3}mol^{-1}cm^{-1} 113200), 239 (133600), 276 (76400), 330 (75400); IR (KBr): ν_{max}/cm^{-1} 3416 (NH), 1626 (C=O), 1588 (C=O), 1560, 1536, 1466, 1351, 1324, 1215, 1183, 1151, 980, 796; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.93 (s, 3H, OMe), 3.96 (s, 3H, OMe),3.98 (s, 3H, OMe), 4.12 (s, 3H, OMe), 6.51 (s, 1H, H5), 6.57 (s, 1H, H5), 7.25 (d, J = 2.2 Hz, 1H, H2), 7.29 (d, J = 2.2 Hz, 1H, H2), 7.44–7.64 (m, 8H, ArH), 9.93 (s, 1H, NH), 10.88 (s, 1H, NH), 11.44 (s, 1H, NH), 11.55 (d, J = 1.5 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.5, 55.9, 57.2, 57.3 (OMe), 88.4, 88.7 (C5), 96.2, 101.6 (C7), 109.9, 110.2 (C3), 115.4, 116.4,118.6, 118.8 (ArC), 123.6, 123.7 (C2), 130.4, 130.5,

131.1, 131.1 (ArCH), 134.7, 135.0, 137.1, 138.1, 156.6, 157.2, 161.0, 162.0 (ArC), 164.6, 165.8, 187.7 (C=O); HRMS (+ESI): found *m/z* 775.0406 ($[M+H]^+$), $[C_{35}H_{28}Br_2N_4O_7H]^+$ requires *m/z* 775.0398 (monoisotopic mass).

3–(4–Chlorophenyl)–*N′*–(2–(3–(4–chlorophenyl)–4,6–dimethoxy–1*H*–indol–7–yl)–2–oxoacetyl)–4,6–dimethoxy–1*H*–indole–7–carbohydrazide (15b). Dark yellow solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 82500), 237 (95700), 275 (59900); IR (KBr): ν_{max}/cm^{-1} 3418 (NH), 1626 (C=O), 1583 (C=O), 1560, 1536, 1465, 1354, 1324, 1215, 1183, 1152, 1090, 980, 796; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.93 (s, 3H, OMe), 3.95 (s, 3H, OMe), 3.98 (s, 3H, OMe), 4.12 (s, 3H, OMe), 6.51 (s, 1H, H5), 6.57 (s, 1H, H5), 7.24 (d, *J* = 2.1 Hz, 1H, H2), 7.29 (d, *J* = 2.3 Hz, 1H, H2), 7.32–7.72 (m, 8H, ArH), 9.94 (s, 1H, NH), 10.89 (s, 1H, NH), 11.44 (s, 1H, NH), 11.55 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.5, 55.8, 57.2, 57.3 (OMe), 88.4, 88.7 (C5), 96.2, 101.6 (C7), 109.9, 110.2 (C3), 115.4, 116.3 (ArC), 123.6, 123.7 (C2), 127.5, 127.6 (ArCH), 130.1, 130.4 (ArC), 130.7, 130.7 (ArCH),134.3, 134.7, 137.0, 138.1, 156.6, 157.2, 161.0, 162.0 (ArC), 164.6, 165.9, 187.7 (C=O); HRMS (+ESI): found *m/z* 687.1406 ($[M+H]^+$), $[C_{35}H_{28}Cl_2N_4O_7H]^+$ requires *m/z* 687.1408 (monoisotopic mass).

***N′*–(2–(3–(4–Bromophenyl)–4,6–dimethoxy–1*H*–indol–7–yl)–2–oxoacetyl)–3–(4–chlorophenyl)–4,6–dimethoxy–1*H*–indole–7–carbohydrazide (15c).** Pale yellow solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 201800), 237 (223700), 275 (126900), 330 (120100); IR (KBr): ν_{max}/cm^{-1} 3418 (NH), 1627 (C=O), 1589 (C=O), 1560, 1537, 1465, 1352, 1324, 1215, 1184, 1151, 1089, 980, 796;^{1}H NMR (300 MHz, DMSO–d_6): δ 3.93 (s, 3H, OMe), 3.96 (s, 3H, OMe), 3.98 (s, 3H, OMe), 4.12 (s, 3H, OMe), 6.51 (s, 1H, H5), 6.57 (s, 1H, H5), 7.24 (d, *J* = 2.4 Hz, 1H, H2), 7.28 (d, *J* = 2.4 Hz, 1H, H2), 7.35–7.62 (m, 8H, ArH), 9.93 (s, 1H, NH), 10.88 (s, 1H, NH), 11.43 (d, *J* = 1.6 Hz, 1H, NH), 11.55 (d, *J* = 2.0 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.5, 55.9, 57.2, 57.3 (OMe), 88.4, 88.7 (C5), 96.2, 101.6 (C7), 109.9, 110.2 (C3), 115.4, 116.4, 118.8 (ArC), 123.6, 123.7 (C2), 127.5 (ArCH), 130.1 (ArC), 130.5, 130.7, 131.1 (ArCH), 134.7, 137.1, 138.1, 156.6, 157.2, 161.0, 162.0 (ArC), 164.6, 165.8, 187.7 (C=O); HRMS (+ESI): found *m/z* 731.0900 ($[M+H]^+$), $[C_{35}H_{28}BrClN_4O_7H]^+$ requires *m/z* 731.0903 (monoisotopic mass).

3–(4–Bromophenyl)–*N′*–(2–(3–(4–chlorophenyl)–4,6–dimethoxy–1*H*–indol–7–yl)–2–oxoacetyl)–4,6–dimethoxy–1*H*–indole–7–carbohydrazide (15d). Pale yellow solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 201200), 238 (229000), 275 (134700), 330 (133000); IR (KBr): ν_{max}/cm^{-1} 3416 (NH), 1627 (C=O), 1589 (C=O), 1562, 1537, 1465, 1353, 1324, 1215, 1184, 1151, 1089, 980, 796; ^{1}H NMR (300 MHz, DMSO–d_6): δ3.93 (s, 3H, OMe), 3.96 (s, 3H, OMe),3.98 (s, 3H, OMe), 4.12 (s, 3H, OMe), 6.51 (s, 1H, H5), 6.57 (s, 1H, H5), 7.24 (d, *J* = 2.4 Hz, 1H, H2), 7.29 (d, *J* = 2.5 Hz, 1H, H2), 7.35–7.63 (m, 8H, ArH), 9.93 (d, *J* = 1.8 Hz, 1H, NH), 10.88 (d, *J* = 2.0Hz, 1H, NH), 11.44 (d, *J* = 1.7 Hz, 1H, NH), 11.55 (d, *J* = 1.9 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.5, 55.9, 57.2, 57.3 (OMe), 88.4, 88.7 (C5), 96.2, 101.6 (C7), 109.9,110.2(C3), 115.4, 116.3, 118.6 (ArC), 123.6,123.7 (C2), 127.6 (ArCH), 130.4 (ArC), 130.4, 130.7, 131.1 (ArCH), 134.3, 135.0, 137.0, 138.1, 156.6, 157.2, 161.0, 161.9 (ArC), 164.6, 165.9, 187.7 (C=O); HRMS (+ESI): found *m/z* 731.0902 ($[M+H]^+$), $[C_{35}H_{28}BrClN_4O_7H]^+$ requires *m/z* 731.0903 (monoisotopic mass).

***N′*–(2–(1*H*–indol–3–yl)–2–oxoacetyl)–3–(4–chlorophenyl)–4,6–dimethoxy–1*H*–indole–2–carbohydrazide (19a).** Pale brown solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 70000), 253 (41400), 323 (28600); IR (KBr): ν_{max}/cm^{-1} 3325 (NH), 3278 (NH), 1712 (C=O), 1620 (C=O), 1580 (C=O), 1537, 1483, 1425, 1262, 1213, 1138, 816, 747; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.62 (s, 3H, OMe), 3.80 (s, 3H, OMe), 6.19 (d, *J* = 1.6 Hz, H5′), 6.56 (d, *J* = 1.6 Hz, H7′), 7.22–7.33 (m, 2H, H5, H6), 7.34–7.42 (m, 2H, ArH phenyl), 7.42–7.50 (m, 2H, ArH phenyl), 7.51–7.62 (m, 1H, H7), 8.16–8.27 (m, 1H, H4), 8.64 (d, *J* = 3.1 Hz, 1H, H2), 9.41 (s, 1H, NH indole), 10.60 (bs, 1H, NH indole), 11.69 (s, 1H, NH hydrazide), 12.31 (d, *J* = 2.6 Hz, 1H, NH hydrazide); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.0, 55.3 (OMe), 86.7, 92.6 (ArCH), 111.2, 112.6 (ArC), 112.7 (ArCH), 118.5 (ArC), 121.2, 122.7, 123.6 (ArCH), 123.8, 125.7 (ArC), 126.9 (ArCH), 131.1 (ArC), 132.9 (ArCH), 133.4, 136.5, 137.6 (ArC), 138.4 (ArCH), 155.1, 158.6 (ArC), 160.7, 163.4, 182.2 (C=O); HRMS (+ESI): found *m/z* 539.1110 ($[M+Na]^+$), $[C_{27}H_{21}ClN_4O_5Na]^+$ requires *m/z* 539.1093 (monoisotopic mass).

***N′*–(2–(1*H*–indol–3–yl)–2–oxoacetyl)–3–(4–bromophenyl)–4,6–dimethoxy–1*H*–indole–2–carbohydrazide (19b).** Creamy white solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 100400), 254 (62300), 321 (43200); IR (KBr): ν_{max}/cm^{-1} 3325 (NH), 3270(NH), 1708 (C=O), 1619 (C=O), 1578 (C=O), 1537, 1483, 1425, 1262, 1212, 1138, 817, 746; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.62 (s, 3H, OMe), 3.80 (s, 3H, OMe), 6.19 (d, *J* = 1.9 Hz, H5′), 6.55 (d, *J* = 1.9 Hz, H7′), 7.23–7.33 (m, 2H, H5, H6), 7.36–7.43 (m, 2H, ArH phenyl), 7.47–7.53 (m, 2H, ArH phenyl), 7.53–7.59 (m, 1H, H7), 8.16–8.25 (m, 1H, H4), 8.64 (d, *J* = 3.3 Hz, 1H, H2), 9.42 (s, 1H, NH indole), 10.60 (s, 1H, NH indole), 11.68 (s, 1H, NH hydrazide), 12.30 (d, *J* = 3.0 Hz, 1H, NH hydrazide); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.0, 55.3 (OMe), 86.7, 92.7 (ArCH), 111.1, 112.6 (ArC), 112.7 (ArCH), 118.5, 119.8 (ArC), 121.2, 122.7, 123.6 (ArCH), 123.8, 125.7 (ArC), 129.8, 133.3 (ArCH), 133.8, 136.5, 137.6 (ArC), 138.4 (ArCH), 155.1, 158.6 (ArC), 160.7, 163.4, 182.3 (C=O); HRMS (+ESI): found *m/z* 583.0604 ($[M+Na]^+$), $[C_{27}H_{21}BrN_4O_5Na]^+$ requires *m/z* 583.0588 (monoisotopic mass).

***N′*–(2–(1*H*–indol–3–yl)–2–oxoacetyl)–4,6–dimethoxy–3–(4–methoxyphenyl)–1*H*–indole–2–carbohydrazide (19c).** Pale brown solid; mp > 300 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 60300), 251 (39800), 317 (26400); IR (KBr): ν_{max}/cm^{-1} 3324 (NH), 3271 (NH), 1709 (C=O), 1614 (C=O), 1582 (C=O), 1542, 1482, 1426, 1287, 1239, 1211, 1150, 1138, 813, 746; ^{1}H NMR (300 MHz, DMSO–d_6): δ3.59 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.80 (s, 3H, OMe), 6.15 (d, *J*

= 1.9 Hz, H5′), 6.54 (d, *J* = 1.9 Hz, H7′), 6.90–6.99 (m, 2H, ArH phenyl), 7.23–7.33 (m, 2H, H5, H6), 7.34–7.43 (m, 2H, ArH phenyl), 7.51–7.60 (m, 1H, H7), 8.15–8.25 (m, 1H, H4), 8.64 (d, *J* = 3.2 Hz, 1H, H2), 8.68 (s, 1H, NH indole), 10.60 (bs, 1H, NH indole), 11.59 (s, 1H, NH hydrazide), 12.32 (d, *J* = 2.9 Hz, 1H, NH hydrazide); ^{13}C NMR (75 MHz, DMSO–d_6): δ 55.0, 55.0, 55.2 (OMe), 86.6, 92.4 (ArCH), 111.8, 112.5 (ArC), 112.6, 112.8 (ArCH), 119.5 (ArC), 121.2, 122.7 (ArCH), 123.2 (ArC), 123.6 (ArCH), 125.7, 126.5 (ArC), 132.1 (ArCH), 136.5, 137.7 (ArC), 138.4 (ArCH), 155.4, 158.2, 158.6 (ArC), 160.8, 163.2, 182.0 (C=O); HRMS (+ESI): found *m/z* 535.1601 ([M+Na]$^+$), [$C_{28}H_{24}N_4O_6Na$]$^+$ requires *m/z* 535.1588 (monoisotopic mass).

General procedure for the synthesis of indolyl–oxoacetohydrazides 16–18

The 7–glyoxyloyl chloride **6a–b** or 3–glyoxyloyl chloride **9** (1.0 equiv) was dissolved in anhydrous acetonitrile. Hydrazine hydrate (0.5 equiv for **16** and **18**, excess for **17**) was added followed by triethylamine (10 drops) and the solution was stirred at room temperature for 1.5 h **(16** and **18)** or 5 h **(17)**. The reaction mixture was quenched with ice–water and the resulting precipitate was filtered, dried and recrystallized from methanol to yield the product as a yellow solid at 50–76% yield.

2–(3–(4–Bromophenyl)–4,6–dimethoxy–1*H*–indol–7–yl)–*N*′–(2–(3–(4–bromophenyl)–4,6–dimethoxy–1*H*–indol–7–yl)–2–oxoacetyl)–2–oxoacetohydrazide (16). Yellow solid; mp 277–279 ºC (methanol); UV–vis: λ_{max}(THF)/nm 212 (ε/dm^{3}mol^{-1}cm^{-1} 50700), 331 (18800); IR (KBr): ν_{max}/cm^{-1} 3396 (NH), 1610 (C=O), 1581 (C=O), 1557, 1534, 1323, 1217; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.95 (s, 6H, OMe), 3.97 (s, 6H, OMe), 6.49 (s, 2H, H5), 7.24 (d, *J* = 2.5 Hz, 2H, H2), 7.46–7.57 (m, 8H, ArH), 10.52 (s, 2H, NH), 11.56 (d, *J* = 2.4 Hz, 2H, NH); ^{13}C NMR (75 MHz, DMSO–d_6): δ 56.2, 57.3 (OMe), 88.9 (C5), 101.7 (C7), 110.2 (C3), 116.7, 119.2 (ArC), 124.0 (C2), 130.9, 131.5 (ArCH), 135.0, 137.5, 161.4, 162.3, 167.0 (ArC), 179.5, 188.3 (C=O); HRMS (+ESI): found *m/z* 803.0347 ([M+H]$^+$), [$C_{36}H_{28}Br_2N_4O_8H$]$^+$ requires *m/z* 803.0347 (monoisotopic mass).

2–(3–(4–Chlorophenyl)–4,6–dimethoxy–1*H*–indol–7–yl)–2–oxoacetohydrazide (17). Pale yellow solid; mp 200–202 ºC (methanol); UV–vis: λ_{max}(THF)/nm 213 (ε/dm^{3}mol^{-1}cm^{-1} 87400), 230 (82200), 254 (85900), 330 (52600); IR (KBr): ν_{max}/cm^{-1} 3325 (NH), 1612 (C=O), 1580 (C=O), 1560, 1537, 1326, 1257, 1220, 1093, 799; ^{1}H NMR (300 MHz, DMSO–d_6): δ 3.90 (s, 3H, OMe), 3.93 (s, 3H, OMe), 4.34 (bs, 2H, NH_2), 6.46 (s, 1H, H5), 7.20 (d, *J* = 2.5 Hz, 1H, H2), 7.35–7.44 (m, 2H, ArH), 7.48–7.58 (m, 2H, ArH), 9.40 (s, 1H, NH), 11.51 (d, *J* = 1.8 Hz, 1H, NH); HRMS (+ESI): found *m/z* 396.0734 ([M+Na]$^+$), [$C_{18}H_{16}ClN_3O_4Na$]$^+$ requires *m/z* 396.0722 (monoisotopic mass). The compound was not soluble enough in DMSO–d_6 for ^{13}C NMR measurement.

***N*′–(2–(1*H*–indol–3–yl)–2–oxoacetyl)–2–(1*H*–indol–3–yl)–2–oxoacetohydrazide (18).** Pale yellow solid; mp (decomp) > 350 ºC (methanol) *lit*[30]; IR (KBr): ν_{max}/cm^{-1} 3229 (NH), 1597 (C=O), 1433, 1237, 1144, 928, 743; ^{1}H NMR (300 MHz, DMSO–d_6): δ 7.22–7.38 (m, 4H, H5, H6), 7.52–7.64 (m, 2H, H7), 8.19–8.33 (m, 2H, H4), 8.71 (s, 2H, H2), 10.75 (s, 2H, NH indole), 12.37 (s, 2H, NH hydrazide); ^{13}C NMR (75 MHz, DMSO–d_6): δ 112.5 (ArC), 112.7, 121.3, 122.8, 123.7 (ArCH), 125.8, 136.5 (ArC), 138.5 (ArCH), 163.1, 181.8 (C=O); HRMS (+ESI): found *m/z* 397.0916 ([M+Na]$^+$), [$C_{20}H_{14}N_4O_4Na$]$^+$ requires *m/z* 397.0907 (monoisotopic mass). The compound was not soluble enough in THF for UV–vis measurement.

Biological assays

ELISA

Full–length σ^A was overproduced, purified[25] and diluted to 250 nM in phosphate buffered saline (PBS). 100 µl of the solution was added into NUNC Maxisorp™ microtitre plate wells, followed by overnight incubation at 4 °C. The wells were washed three times with 300 µl of PBS and blocked by incubating 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). 400 nM purified GST tagged RNAP β′ subunit fragment[26] in 50 µl PBS was mixed with 30 µM compounds in 50 µl PBS at 37 °C for 10 min, and then added to wells followed by incubation at room temperature for 1 h. Wells were washed three times with 300µl of PBS/Tween–20 wash buffer, 100 µl of rabbit anti–GST primary antibody (1:2000 in PBS) was added to each well and the wells were incubated at room temperature for 1 h. Wells were washed three times with 300 µl of PBS/Tween–20 wash buffer. HRP–conjugated goat–anti–rabbit secondary antibody (1:2000 in PBS) was added to each well and the wells were incubated at room temperature for 1 h. 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 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. Samples were tested in triplicate and the absorbance of each sample was compared to the control without exposure to compounds to calculate absolute inhibition percentages.

Growth inhibition assays

Compounds were dissolved at 50 mM in DMSO and then diluted to 200 µM in 100 µl of Luria–Bertani (LB) medium into individual wells in a 96–well plate. *E. coli* DH5α or *B. subtilis* 168 cells were grown at 37 °C in 5 ml LB with shaking until the OD600 reached 0.6–0.7, and 5 µl of the culture was added to each well. The plate was incubated in a FLUOstar Optima plate reader (BMG Labtech) at 37 °C shaking at 600 rpm. The OD600 of the culture was taken every 10 min over a 16 h period using LB as the blank. Samples were tested in triplicate and the growth pattern of each sample was compared to cells exposed to equal amounts of DMSO.

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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