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Development of novel isatin thiazolyl-pyrazoline hybrids as promising antimicrobials in MDR pathogens†

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Microbial Multidrug Resistance (MDR) is an emerging global crisis. Derivatization of natural or synthetic scaffolds is among the most reliable strategies to search for and obtain novel antimicrobial agents for the treatment of MDR infections. Here, we successfully manipulated the synthetically flexible isatin moieties to synthesize 22 thiazolyl-pyrazolines hybrids, and assessed their potential antimicrobial activities *in vitro* against various MDR pathogens, using the broth microdilution calorimetric XTT reduction method. We chose 5 strains to represent the major MDR microorganisms, *viz*: Methicillin-resistant *S. aureus* (MRSA), and Vancomycin-resistant *E. faecalis* (VRE) as Gram-positive bacteria; Carbapenem-resistant *K. pneumonia* (CRKP), and Extended-spectrum beta-lactamase *E. coli* (ESBL-E), as Gram-negative bacteria; and Fluconazole-resistant *C. albicans* (FRCA), as a yeast-like unicellular fungus. The cytotoxicity of compounds **9f** and **10h** towards mammalian lung fibroblast (MRC-5) cells demonstrated their potential satisfactory safety margin as represented by their relatively high IC_{50} values. The target compounds showed promising anti-MDR activities, suggesting they are potential leads for further development and *in vivo* studies.

1 Introduction

Since their discovery, antimicrobial agents have been a reliable weapon in fighting life-threatening infections. However, some pathogens are naturally resistant to some antimicrobials, and several other microorganisms have become resistant through natural genetic selection and/or passing the resistance factor(s) from one organism to another, thus propagating the resistance(s) between different organisms.[1] The excessive and irresponsible use of antimicrobials has played a major role in an emerging global crisis of drug resistance. Additionally, the noticeable lack of investment in new drug discovery efforts by pharma – due to low profits and harsh regulations – has exacerbated the problem.[2]

Multidrug resistance (MDR) is the acquired insensitivity of a microorganism toward several structurally distinct classes of antimicrobials that have different molecular targets.[3] Although MDR usually evolves in nosocomial infections, several have become a widespread cause of public acquired infections.[4] Opportunistic pathogenic yeast *Candida albicans*; Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*; as well as Gram-positive Vancomycin-resistant enterococcus (VRE) and Methicillin-resistant *Staphylococcus* aureus (MRSA) are amongst the well-known microorganisms capable of developing MDR.[4] By 2050, mortality rates from infections are expected to rise significantly, and it is estimated that 10 million people will be at risk of premature death if this problem has not been addressed.[5] Therefore, it's crucial to develop new antimicrobial agents with potent activity against MDR pathogens.

Strategies, to obtain new antimicrobial agents for the treatment of MDR infections, range from the production of analogs

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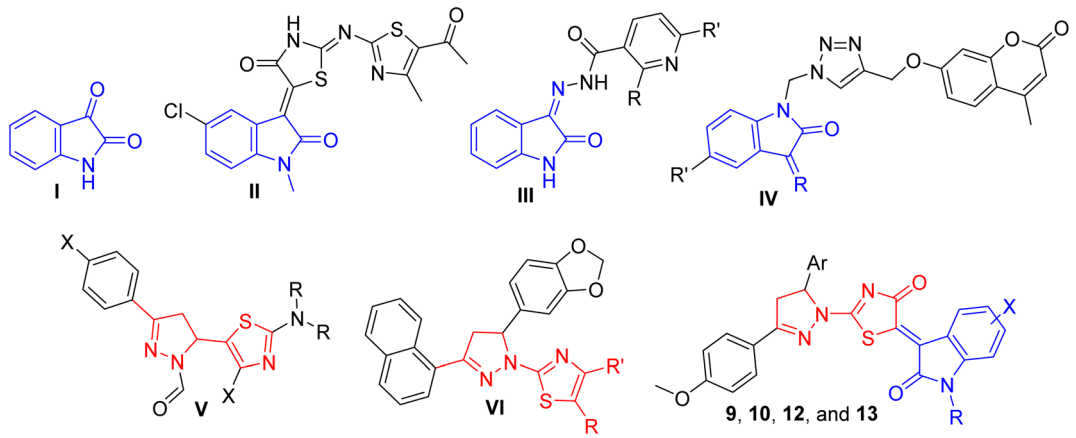


Fig. 1 Chemical structure of isatin(I), antimicrobial isatin derivatives (II–IV), antimicrobial thiazolyl-pyrazoline derivatives, as well as the compounds reported in this manuscript **9a–h**, **10a–h**, **12a–c**, and **13a–c**.

or derivatives of existing drug molecules to the discovery of novel natural or synthetic scaffolds.[6] Isatin (**I**, Fig. 1) is a well-known heterocyclic natural compound that can be found in the human body as a metabolite of adrenaline, as well as in different plants such as *Isatis* and *Couroupita* genera.[7] The synthetic flexibility of the isatin building block has prompted its broad implementation in targeted derivatization to generate novel pharmacologically active molecules.[7] For example, isatin-derived Sunitinib is a multi-targeted receptor tyrosine kinase inhibitor that has been approved by the FDA for the treatment of various tumours.[8] In addition, many isatin derivatives have shown antiprotozoal,[9] antiviral,[10] and anti-cancer activities.[9,11–13]

In a previous work, we reported that the hybrid structures from combining thiazolylamino–thiazolidinone with isatin motifs are a successful approach to developing a promising scaffold with good antimicrobial and anti-mycobacterial activities. In particular, compound **II** (Fig. 1) demonstrated promising activity toward both VRE and MRSA (MIC = 7.81 and 3.90 mg ml^{-1}, respectively).[14] In another recent study, we reported the potent activity of isatin-based nicotinohydrazide derivatives **III** (Fig. 1) against bronchitis causing *K. pneumonia* as well as against isoniazid/streptomycin-resistant *Mycobacterium tuberculosis*.[15] Similarly, isatin–triazole–coumarin hybrids **IV** (Fig. 1) showed promising activity against *M. tuberculosis*.[16]

Other heterocyclic compounds with electron-rich nitrogen and/or sulfur atoms are also recognized for their pharmacological activities. They are widely distributed in nature as alkaloids, vitamins, and glycosides.[17] Pyrazolines, for instance, possess antimicrobial, anti-tubercular, anticancer, and anti-inflammatory activities.[18,19] Notably, the replacement of the β-lactam ring with a pyrazolidinone ring in penicillins has retained the antibiotic activity.[20] Similarly, thiazoles, have exhibited antimicrobial,[21,22] anti-tuberculosis,[23] and antiviral activities.[24,25] Remarkably, compounds that contain thiazolyl-pyrazolines (**V**, Fig. 1) moieties showed effective inhibition against methicillin-susceptible/-resistant *S. aureus*, and vancomycin-intermediate *S. aureus*.[26] In addition, a variety of derivatives demonstrated diverse biological activities such as antimicrobial (**VI**, Fig. 1),[17,27] antiprotozoal[28,29] anti-proliferative,[27,30,31] and anti-Alzheimer[32] activities.

In this study, we describe the design, synthesis, and biological evaluation of a novel set of isatin thiazolyl-pyrazolines hybrid small molecules (**9a–h**, **10a–h**, **12a–c**, and **13a–c**, Fig. 1) as potential antimicrobial agents against various MDR pathogens. The antimicrobial activities of the newly synthesized molecules are screened *in vitro*, using the broth microdilution calorimetric XTT reduction method, against 5 MDR strains *viz*; Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus faecalis* (VRE), Carbapenem-resistant *Klebsiella pneumonia* (CRKP), Extended-spectrum beta-lactamase *Escherichia coli* (ESBL-E), and Fluconazole-resistant *Candida albicans*.

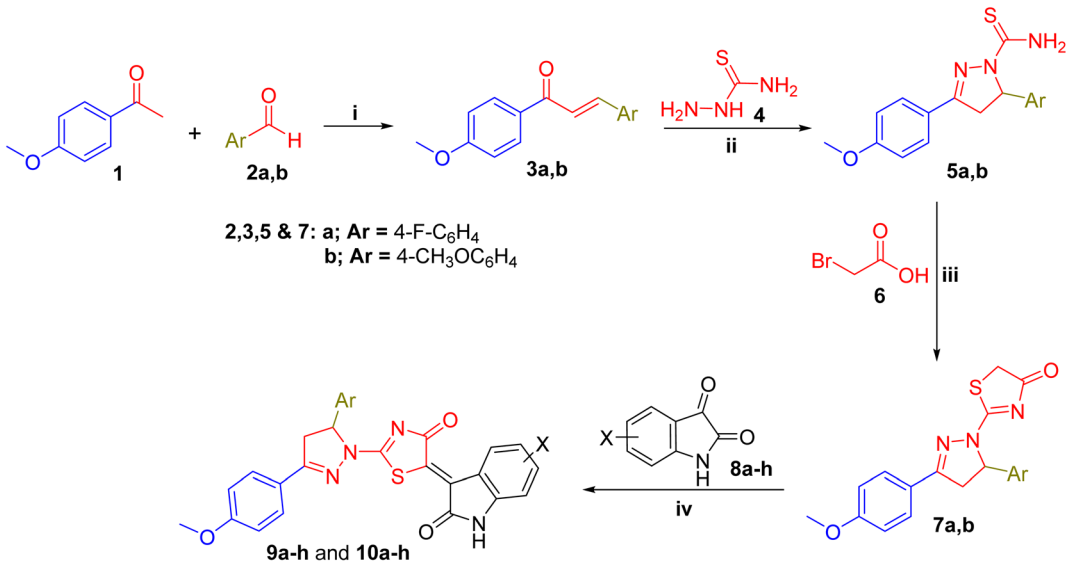
2. Results and discussion

2.1. Chemistry

The synthetic methodology adopted to obtain target compounds (**9a–h**, **10a–h**, **12a–c**, and **13a–c**) mainly involved straightforward condensation and/or cycloaddition chemistry (Schemes 1 and 2). Firstly, chalcone derivatives **3a-b** were synthesized by a simple Claisen–Schmidt condensation reaction of the precursor benzaldehyde derivatives (**2a-b**) each with *p*-methoxyacetophenone (Scheme 1).

The positive mesomeric effect exerted by the *p*-methoxy group in *p*-methoxyacetophenone may account for the relatively low acidity of the α-CH_3 group and harsher enolization conditions (40% KOH) in this reaction.

Treatment of chalcones **3a-b** with thiosemicarbazide (**4**) afforded pyrazoline derivatives **5a-b**. The reaction proceeds regioselectively through a semicarbazone intermediate, formed by nucleophilic attack of the hard thiosemicarbazide N1 nucleophilic site to the hard carbonyl electrophilic site (see the Hard and Soft Acid and Base (HSAB) theory[33]). This rapidly cyclizes to afford the desired pyrazolines **5a-b** by intramolecular addition reaction of the softer nucleophilic end (thiosemicarbazone N2) to the softer electrophilic end (β-carbon of



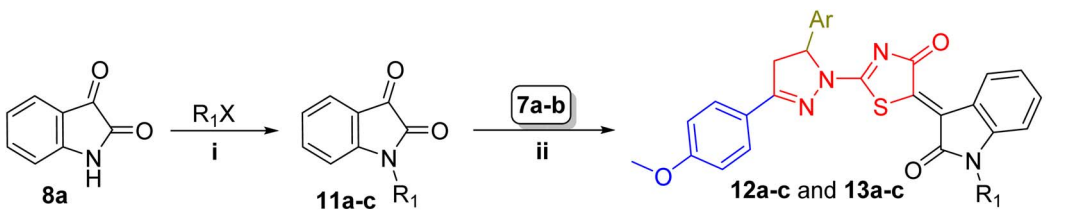
9	Ar	X
a	4-F-C_6H_4	H
b		5-F
c		7-F
d		5-Cl
e		7-Cl
f		5-OCH_3
g		5-CH_3
h		5,7-$(CH_3)_2$

10	Ar	X
a	4-$CH_3OC_6H_4$	H
b		5-F
c		7-F
d		5-Cl
e		7-Cl
f		5-OCH_3
g		5-CH_3
h		5,7-$(CH_3)_2$

Scheme 1 *Reagent and conditions:* (i) KOH (40%), ethanol/water 1 : 1, stirring 4 h, rt; (ii) NaOH (40% aq) (4 equiv.)/ethanol, reflux 3 h; (iii) NaOAc (2 equiv.)/AcOH, reflux 5 h; (iv) NaOAc (2 equiv.)/AcOH, reflux 3 h.

the α,β-unsaturated C=N). Overall, only one regioisomer of the pyrazoline nucleus is formed (**5a-b**). The reaction requires base catalysis (40% aqueous NaOH) likely for activation of thiosemicarbazone N2 *via* deprotonation prior to intramolecular addition. It is noteworthy that the sp3 carbon atom (pyrazoline C5) formed during this reaction is asymmetric. Whilst the reaction is not enantioselective and the formation of **5a-b** leads to a racemic mixture, the adjacent methylene group hydrogens (at pyrazoline C4) are now diastereotopic. This feature was clearly found in the splitting pattern of pyrazoline C4 protons in compounds **5**, **7**, **9**, **10**, **12** and **13** in the NMR spectra. Mutual second-order geminal, as well as vicinal (*trans* or *cis*) coupling, were observed (see 1H NMR charts of **9**, **10**, **12** and **13** in the ESI†) between C4 protons and with C4–C5 protons *J* values as high as 18 (geminal at C4) and 11 or 4 Hz (vicinal *trans* or *cis*), respectively.

Similarly, the free ambident nucleophilic 1-carbothioamide moiety of **5a-b** was utilized in a second [3 + 2] cyclization reaction; here with bromoacetic acid (**6**) as the ambident electrophile (Scheme 1). Again, by applying the HSAB theory, the 'softer' nucleophilic site (sulfur of **5**) reacts regioselectively with the 'softer' electrophilic site (α-bromo-substituted carbon of **6**). This afforded the desired pyrazolin-1-ylthiazol-4(5*H*)-ones (**7a-b**). Due to the low electrophilicity of the COOH group (resulting



12	Ar	R_1
a	4-F-C_6H_4	Ethyl
b		Allyl
c		Isobutyl

13	Ar	R_1
a	4-$CH_3OC_6H_4$	Ethyl
b		Allyl
c		Isobutyl

Scheme 2 *Reagent and conditions:* (i) K_2CO_3 (2 equiv.), KI (0.2 equiv.)/DMF, stirring 6 h, 80 °C; (ii) NaOAc (2 equiv.)/AcOH, reflux 3 h.

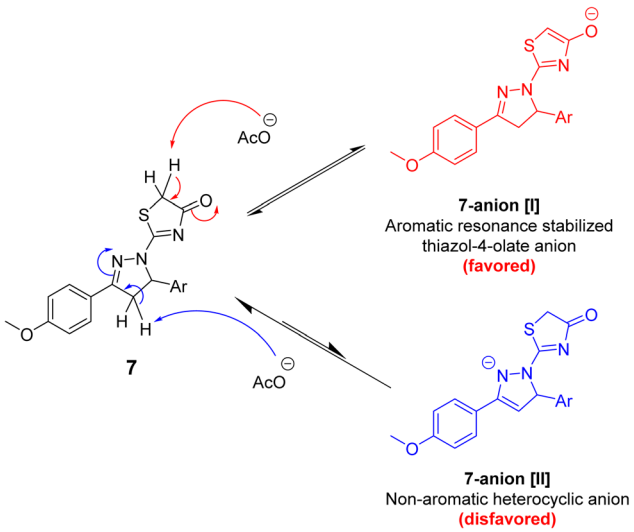
from the poor leaving group ability of OH), the reaction required a longer reflux period in dehydrating conditions (glacial acetic acid). A similar cyclization reaction was previously reported to afford oxazol-4(5*H*)-one nucleus (instead of thiazol-4(5*H*)-one) from the precursor hippuric acid derivative (carbamide derivative instead of carbothioamide illustrated herein) showing similar regioselectivity, while requiring harsher conditions (acetic anhydride instead of acetic acid).[34] Both compound series **5a-b** and **7a-b** were previously reported (see the Experimental section for further details).

Both N1-unsubstituted and N1-substituted isatin derivatives were utilized for the synthesis of final compounds, (**9/10a–h**) and (**12/13a–c**) series respectively. The N1-substituted isatin derivatives were prepared by N1-alkylation of isatin (**8a**) *via* SN2 reaction with the corresponding alkyl halide (R1X, Scheme 2). For this reaction, primary alkyl halides (including the reactive allyl bromide), anhydrous potassium carbonate (as a base), and a polar aprotic solvent (DMF) were utilized to enhance the SN2 reaction conditions. It is noteworthy that the reaction rate could be enhanced as a result of adding a catalytic amount of potassium iodide as a nucleophilic catalyst (Finkelstein reaction) as has been demonstrated recently by our group.[35] This reaction afforded N1-alkyl isatin derivatives **11a–c** which were previously reported (see the Experimental section for further details).

Finally, target compound series (**9/10a–h**) and (**12/13a–c**) were synthesized by Knoevenagel condensation of the active methylene group within the pyrazolin-1-ylthiazol-4(5*H*)-one derivatives (**7a-b**) with the ketonic C=O group of the isatin derivatives **8a–h** and **11a–c**, respectively. The reaction was carried out in dehydrating conditions (refluxing glacial acetic acid) and reached completion within a relatively short period (3 h) by using a mild solvent-compatible base (sodium acetate). Analysis of the NMR spectra of the products confirms that the active methylene group at C4 of the pyrazoline ring, rather than that at C5 of thiazol-4(5*H*)-one (within the 7 nucleus, Scheme 1), is involved in the condensation reaction.

The ^{1}H NMR charts of compound series (**9/10a–h** and **12/13a–c**) (ESI†) clearly show that the pyrazoline ring was intact (showing geminal coupling of $J = 18$ Hz for the diastereotopic C4 protons at $\delta = 3.4$–4.2 ppm, as discussed earlier) while the C5 methylene of thiazol-4(5*H*)-one is absent which conclusively shows the latter to be involved in the Knoevenagel condensation. Analogous activation of the C5 methylene group of oxazol-4(5*H*)-one derivative (closely related to thiazol-4(5*H*)-one) was carried out at similarly mild conditions (sodium acetate base at low temperature) to allow its coupling with diazonium salt (reactive electrophile).[34]

The regioselective Knoevenagel condensation at the thiazol-4(5*H*)-one (rather than pyrazoline) ring of **7** series is explained in terms of relatively easier enolization of thiazol-4(5*H*)-one nucleus (by deprotonation of C5 methylene group) affording stable **7-anion [I]** unlike the alternative deprotonation of pyrazoline C4 which affords the less stable **7-anion [II]**, Scheme 3. The relatively enhanced **7-anion [I]** stability difference is likely attributed to its aromatic nature, the strong negative mesomeric effect of the exocyclic carbonyl at C4 alongside sulfur atom of the thiazol-4(5*H*)-one ring being able to stabilize an adjacent



Scheme 3 Structure and stability of **7-anions** reflecting the relative ease of their formation upon treating **7** with a mild base and providing evidence of the regioselective Knoevenagel condensation at the thiazol-4(5*H*)-one ring to afford target compounds.

negative charge *via* accepting electrons in its higher vacant orbitals,[36] as shown in the canonical resonating structures outlined in Scheme 3.

Compounds (**9/10a–h** and **12/13a–c**) were fully characterised, their structures were confirmed by ^{1}H and ^{13}C NMR (See the NMR reports in the Experimental section and the spectral charts in the ESI†), and their purity was assessed by HRMS and/or CHN elemental analysis.

2.2. Biological evaluation

2.2.1. Antibacterial activities. The antibacterial activity of the synthesized isatin derivatives was screened *in vitro*, using the broth microdilution calorimetric XTT reduction method, against 4 MDR strains *viz*; Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 700788) and Vancomycin-resistant *Enterococcus faecalis* (VRE, BAA-2365) strains as examples of Gram-positive bacteria; Carbapenem-resistant *Klebsiella pneumonia* (CRKP, ATCC BAA-2342) and Extended-spectrum beta-lactamase *Escherichia coli* (ESBL-E, BAA-199) strains to represent Gram-negative bacteria.

Table 1 demonstrates the values of growth inhibition (% at 1.95 μg ml^{-1}) and the minimum inhibitory concentration (MIC, μg ml^{-1}) of the synthesized derivatives (**9/10a–h** and **12/13a–c**) against MDR Gram-positive bacterial strains (MRSA and VRE). Generally, MRSA was more sensitive to the tested derivatives than VRE. Compounds **9f** and **10h** demonstrated 100% inhibition against MRSA at the applied concentration, with MIC of 0.98 μg ml^{-1}. Similarly, compound **10f** showed 100% inhibition, but with almost double the MIC (1.95 μg ml^{-1}). Against VRE, the compounds **9f**, **10h**, and **10f** retained outstanding activities, with percent inhibition ranging between 60–73%. Compound **9f** was the most active with a MIC of 7.80 μg ml^{-1}, which is approximately half that of compounds **10h** and **10f** (MIC of

Table 1 Antibacterial activity of the synthesized isatin derivatives (**9/10a–h** and **12/13a–c**) against MDR Gram-positive strains; expressed as the mean of inhibitory percentages (inhibitory % at 1.95 μg ml^{-1}), and minimum inhibitory concentrations (MIC) in μg ml^{-1}

	% Inhibition with 1.95 μg ml^{-1}		MIC (μg ml^{-1})	
Compound	MRSA	VRE	MRSA	VRE
9a	21.96 ± 1.1	NA	250	NA[a]
9b	89.35 ± 0.74	52.19 ± 2.2	3.9	31.25
9c	41.23 ± 2.2	21.79 ± 2.2	62.5	250
9d	95.17 ± 1.3	61.23 ± 1.4	3.9	31.25
9e	69.17 ± 1.1	32.17 ± 1.3	7.81	125
9f	100.00 ± 0.00	73.25 ± 1.2	0.98	7.81
9g	92.17 ± 0.96	56.31 ± 1.9	3.9	15.63
9h	92.15 ± 0.00	52.17 ± 1.3	3.9	31.25
10a	59.31 ± 1.4	NA	15.63	NA
10b	63.25 ± 1.3	NA	15.63	NA
10c	71.95 ± 1.8	39.27 ± 0.99	7.81	62.5
10d	73.24 ± 0.97	50.64 ± 1.7	7.81	31.25
10e	19.36 ± 0.92	NA	250	NA
10f	100.00 ± 0.00	63.28 ± 0.84	1.95	15.63
10g	82.19 ± 1.3	50.16 ± 0.58	3.9	31.25
10h	100.00 ± 0.00	60.19 ± 1.9	0.98	15.63
12a	71.29 ± 1.1	39.25 ± 1.6	7.81	125
12b	91.35 ± 1.6	53.96 ± 0.85	3.9	62.5
12c	53.21 ± 1.7	23.17 ± 2.2	62.5	500
13a	11.96 ± 1.1	NA	>1000	NA
13b	79.85 ± 1.3	41.96 ± 0.96	3.9	62.5
13c	42.19 ± 0.85	NA	125	NA
Vancomycin	98.84 ± 1.69		1.95	
Linezolid		91.96 ± 2.83		7.81

[a] NA: no activity.

15.63 μg ml^{-1}). Compounds **9b**, **9d**, **9g**, **9h**, **11g**, **12b**, and **13b** exhibited the same MIC (3.90 μg ml^{-1}) against MRSA with percent inhibition that ranged between 80–95%, while against VRE, they showed percent inhibition of 50–60% with relatively higher MIC that ranged between 15.63 to 31.25 μg ml^{-1}. The remaining compounds showed moderate, weak, or no activities against either strain.

It can be inferred from the results that variation in type or position of the substituents has a profound impact on the activity of the derivatives. In the *p*-fluoroaryl series (**9a–h**), the 5-substituent on the isatin ring is critical to the activity. The derivative with a 5-methoxy substitution, compound **9f**, was able to show the most potent activity against both MRSA and VRE. However, derivatives with 5-F (**9b**), 5-Cl (**9d**), 5-methyl (**9g**), or 5,7 dimethyl (**9h**) have reduced the efficacy by about 4 folds. Lack of a substituent on the isatin (**9a**), or shifting the substitution to position 7 (**9c** and **9e**), dramatically diminished the activity. In contrast, in the *p*-methoxyaryl series (**10a–h**), compound **10h** with a 5,7-dimethyl substituent on the isatin motif was the most potent, showing similar activity as compound **9f**. Interestingly though, compound **10f** with the 5-methoxy isatin moiety was still potent although showing a 2-fold increase in the MIC over **9f**. Compound (**10g**) with 5-methyl isatin showed similar potency to **9g**. 5-Halo substituents were significantly less potent mirroring the observation in the *p*-fluoroaryl series **9**. N-substitution on the isatin ring (**12/13a–c**) in general reduced the efficacy, suggesting the importance of the free (NH) for the activity. Anyway, N-substituting with an allyl group (**12b** and **13b**) retained some activity, whereas ethyl (**12a** and **13a**) or isobutyl (**12c** and **13c**) substituents almost abolished the activity.

Table 2 demonstrates the activities against MDR Gram-negative strains (ESBL-E and CRKP). Generally speaking, the compounds are more potent against Gram-negative strains. In the *p*-fluoroaryl series, compound **9f** again demonstrated 100% inhibition against both ESBL-E and CRKP, with MIC of 0.24 μg ml^{-1} and 0.98 μg ml^{-1} respectively. In the *p*-methoxyaryl series **10**, compound **10h** was again potent showing 100% inhibition against ESBL-E and 80% against CRKP, with an impressive MIC of 0.48 μg ml^{-1} against ESBL-E, and 1.95 μg ml^{-1} against CRKP. Compounds **9d**, **9g**, **9h**, **10f**, & **12b** demonstrated 100% inhibition toward ESBL-E, with MIC of 0.98 μg ml^{-1} for compounds **9g** & **10f**, while that for the rest of the compounds being 1.95 μg ml^{-1}. Toward CRKP, the compounds showed 70–80% inhibition with compounds **9d**, **10f**, & **12b** having MIC of 3.9 μg ml^{-1}, while the other two compounds' MIC was 7.81 μg ml^{-1}. Compounds **9b**, **9e**, **10c**, **10d**, **10g**, and **13b** exhibited the same MIC (3.9 μg ml^{-1}) against ESBL-E with percent inhibition that varied between 76–100%. Against CRKP, the latter group of compounds showed percent inhibition of 70–80% with MIC that ranged from 3.9 to 7.81, except for compound **9e** which

Table 2 Antibacterial activity of the synthesized isatin derivatives (**9/10a–h** and **12/13a–c**) against MDR Gram-negative strains; expressed as the mean of inhibitory percentages (inhibitory % at 1.95 μg ml^{-1}), and minimum inhibitory concentrations (MIC) in μg ml^{-1}

	Inhibitory[a] %		MIC (μg ml^{-1})	
Compound	ESBL-*E. coli*	CRKP	ESBL-*E. coli*	CRKP
9a	18.37 ± 1.2	6.21 ± 1.8	500	>1000
9b	100.00 ± 0.00	80.19 ± 1.3	3.9	3.9
9c	36.27 ± 1.9	57.31 ± 1.7	62.5	15.63
9d	100.00 ± 0.00	79.24 ± 1.8	1.95	3.9
9e	76.25 ± 1.7	61.08 ± 0.58	3.9	31.25
9f	100 ± 0.00	100 ± 0.00	0.24	0.98
9g	100 ± 0.00	71.24 ± 0.87	0.98	7.81
9h	100 ± 0.00	71.68 ± 1.1	1.95	7.81
10a	38.19 ± 1.4	17.92 ± 1.6	125	500
10b	52.17 ± 0.58	26.47 ± 1.3	31.25	250
10c	81.74 ± 0.58	69.25 ± 1.6	3.9	7.81
10d	86.25 ± 2.1	71.25 ± 1.8	3.9	7.81
10e	19.68 ± 1.5	12.32 ± 0.89	500	500
10f	100.00 ± 0.00	81.74 ± 1.6	0.98	3.9
10g	91.26 ± 0.82	76.32 ± 0.88	3.9	3.9
10h	100.00 ± 0.00	79.35 ± 0.74	0.48	1.95
12a	69.245 ± 2.1	41.08 ± 1.7	7.81	125
12b	100.00 ± 0.00	83.29 ± 2.1	1.95	3.9
12c	56.39 ± 2.1	32.19 ± 0.74	31.25	250
13a	32.96 ± 0.69	NA	250	NA
13b	81.39 ± 1.6	68.96 ± 1.3	3.9	7.81
13c	43.19 ± 1.8	14.97 ± 0.97	125	500
Colistin	100.00 ± 0.00	97.44 ± 2.18	0.98	1.95

[a] NA: no activity.

declined to 61% inhibition and 31.25 μg ml^{-1} MIC. The remaining compounds showed moderate or weak, to almost non-sensible activities against both strains.

The results indicate that, in the *p*-fluoroaryl series (**9a–h**), compound **9f** showed the highest potency against both ESBL-E and CRKP. Replacing its 5-methoxy with 5-methyl (**9g**), 5,7 dimethyl (**9h**), or with a relative halogen (5-Cl, **9d**), retained similar activities, especially against ESBL-E. Exchanging the 5-Cl with a smaller F-group (**9b**) or shifting to position 7 (**9e**) noticeably decreased the activities. The lack of any substituent on the isatin ring (**9a**) or the presence of a small fluoro substitution on position 7 (**9c**) almost abrogated the activity, proving the importance of 5-isatin substitution for the activity. In the *p*-methoxyaryl series (**10a–h**), compound **10h** with 5,7-dimethyl isatin moiety showed similar activity as compound **9f**, while shifting back to the 5-methoxy isatin (**10f**) maintained significant activity with MIC only doubled. Removing or swapping the isatin substituent with an alkyl or halo group considerably decreased or diminished the activity. Again, substitution on the NH of the isatin ring (**12/13a–c**) generally reduced the efficiency, except for compounds (**12b** & **13b**) which retained considerable activity, emphasising the role of the allyl-substitution in regaining the lost activity due to the isatin-NH substitution.

2.2.2. Antifungal activity. The antifungal activity of the synthesized isatin derivatives was screened *in vitro*, using the broth microdilution calorimetric XTT reduction method, against an MDR strain *viz*; Fluconazole-resistant *Candida albicans* (FRCA, ATCC-MYA-1003A) strain as a yeast-like unicellular fungus.

Table 3 presents the values of % inhibition and MIC of the compounds against Fluconazole-resistant *Candida albicans* (FRCA). Compound **9f** again was the most potent and showed 82% inhibition, with MIC of 3.9 μg ml^{-1}. Compounds **10d**, **10f**, **10h**, & **12b** demonstrated 60–70% inhibition with MIC of 7.81 μg ml^{-1}. Compounds **9b**, **9d**, **9g**, & **9h** exhibited the same MIC (15.63 μg ml^{-1}) with percent inhibition ranging around 60%. The remaining compounds showed moderate, weak, or negligible antifungal activity.

Among the *p*-fluoroaryl derivatives **9**, the 5-methoxy substituted **9f** once more has shown the highest potency as antifungal, roughly the same as that observed for anti-Gram-positive bacteria. Replacing the 5-methoxy with any 5-substituent slightly reduced the activity with about a 4× increase in the MIC. Removing or shifting the substitution to position 7, nearly eliminated the activity. In the *p*-methoxyaryl series **10**, compounds with 5-Cl, 5-methoxy, or 5,7-dimethyl (**10d**, **10f** or **10h**) showed good potency with MIC only doubled compared to **9f**. Removing, replacing the isatin substituent with an alkyl or small F group, or shifting to position 7 considerably abolished the activity. Once again, substitution on the NH, except for allyl compounds (**12b** & **13b**), mostly diminished the activity.

Finally, it is noteworthy that the results confirm the superiority of compound **9f** over the other derivatives as an antifungal as well as broad-spectrum antibacterial activities against Gram-positive and Gram-negative bacteria, which strongly advocates further investigation of this molecule as a promising antimicrobial compound against MDR pathogens.

Table 3 Antifungal activity of the synthesized isatin derivatives (**9/10a–h** and **12/13a–c**) against Fluconazole-resistant *Candida albicans* (ATCC-MYA-1003); expressed as the mean of inhibitory percentages (inhibitory % at 1.95 μg ml^{-1}), and minimum inhibitory concentrations (MIC) in[a] μg ml^{-1}

Compound	Inhibitory %	MIC (μg ml^{-1})
9a	NA	NA
9b	61.87 ± 1.1	15.63
9c	24.16 ± 1.1	250
9d	63.75 ± 2.1	15.63
9e	56.31 ± 1.8	31.25
9f	81.69 ± 1.8	3.9
9g	58.91 ± 0.85	15.63
9h	60.82 ± 0.85	15.63
10a	9.12 ± 0.63	>1000
10b	12.32 ± 1.9	500
10c	51.74 ± 0.82	62.5
10d	60.74 ± 0.73	7.81
10e	NA	NA
10f	69.25 ± 0.58	7.81
10g	36.26 ± 1.4	125
10h	65.73 ± 0.67	7.81
12a	32.19 ± 1.3	125
12b	67.98 ± 1.6	7.81
12c	19.87 ± 1.9	500
13a	NA	NA
13b	46.32 ± 0.58	31.25
13c	NA	NA
Ketoconazole	97.24 ± 2.05	3.9

[a] NA: no activity.

Table 4 Cytotoxicity of compounds **9f** and **10h** towards non-tumorigenic lung fibroblast (MRC-5) cells

Comp	IC_{50} (μM)[a]
9f	45.02 ± 2.5
10h	58.10 ± 3.2
Doxorubicin	37.41 ± 1.7

[a] IC_{50} values are the mean of three separate experiments.

2.2.3. Lack of cytotoxic activity towards mammalian cells. With the aim of exploring the safety profile of our lead compounds **9f** and **10h**, we analyzed their cell growth inhibitory activity in non-tumorigenic lung fibroblast MRC-5 cell line utilizing the SRB assay.[37,38] As indicated in Table 4, both the examined molecules **9f** and **10h** displayed only very weak cytotoxicity with IC_{50} values of 45.02 ± 2.5 and 58.10 ± 3.2 μM, respectively.

3. Conclusions

Microbial resistance emerges through natural selection and/or passing the resistance factor(s) from one organism to another, leading to the global health crisis of Multidrug Resistance (MDR). In this study, we followed the approach of hybridizing the natural scaffold isatin with the synthetic

thiazolyl-pyrazolines to produce 22 novel compounds, and screen them as potential antimicrobials. The *in vitro* screening revealed that MRSA was more sensitive to all tested compounds than VRE. The *p*-fluoroaryl compounds showed relatively high potency against both, as well as against ESBL-E, CRKP, and FRCA. The SAR studies showed that the variation in the type or position of the substituents has a profound impact on the activity of the tested derivatives. In the *p*-fluoroaryl series, the 5-substituent on the isatin ring is critical to the activity. Generally speaking, replacing the 5-methoxy with any 5-substituent maintained the activity but with an about 4-fold increase in the MIC. Removing or shifting the substitution to position 7, nearly abolished the activity. Isatin N-substitution, in general, has a negative impact on the efficacy, showing the importance of the free (NH) for the activity. Remarkably compounds **9f** and **10h** have shown superior potency over all other derivatives, being the most active derivatives as antifungal alongside as well as broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. The cytotoxicity of compounds **9f** and **10h** towards mammalian lung fibroblast (MRC-5) cells demonstrated their potential satisfactory safety margin as represented by their relatively high IC_{50} values ($\sim$50 μM) in this cell line. This strongly supports the potential of these molecules as leads for MDR antimicrobial and further development and *in vivo* studies.

4. Experimental

4.1. Chemistry

4.1.1. General. Melting points were measured with a Stuart melting point apparatus and were uncorrected. The NMR spectra were recorded by Bruker 400 MHz spectrometer operating at 400 MHz (^{1}H) and 101 MHz (^{13}C) using deuterated trifluoroacetic acid or deuterated dimethylsulfoxide (DMSO-d_6) as solvent. Elemental analyses were carried out using Thermo Scientific FLASH 2000 CHNS/O analyzer. Unless otherwise mentioned, all reagents and solvents are commercially available and were used without further purification.

4.1.2. General method for preparation of chalcones 3a-b. Chalcone derivatives **3a-b** were synthesized by stirring *p*-methoxyacetophenone (**1**, 3 g, 20 mmol) with the corresponding aldehydes (**2a-b**, 20 mmol) at room temperature for 4 h in aqueous–ethanolic (1 : 1) potassium hydroxide (40% solution). The precipitate was filtered by suction, washed with water, and finally air-dried for several hours. Chalcones **3a-b** were identified by comparing their measured melting points (105–107 °C and 100–102 °C) with the reported values (107–108 °C (ref. 39) and 102–104 °C (ref. 40)) for **3a** and **3b**, respectively. Yields = 88 and 85% for **3a** and **3b**, respectively.

4.1.3. General method for synthesis of 3,5-diaryl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide derivatives (5a-b). To a stirred ethanolic solution (20 ml) of chalcones **3a-b** (10 mmol) and thiosemicarbazide (**4**, 1.1 g, 12 mmol), sodium hydroxide (0.8 g, 20 mmol) was added. Then, the reaction mixture was heated at reflux for 3 h. After that, the precipitated solid was filtered while hot washed with 70% aqueous ethanol (3 × 5 ml), and finally dried at 90 °C. This afforded pyrazoline-1-carbothioamide derivatives (**5a-b**).[41,42] Compounds **5a-b** were used in the next step with no further purification.

4.1.4. General method for synthesis of 2-(4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4(5*H*)-one derivatives (7a-b). The corresponding pyrazoline-1-carbothioamide derivative (**5**, 7 mmol),-bromoacetic acid (**6**, 1.07 g, 7.7 mmol, 1.1 equiv.) and anhydrous sodium acetate (1.1 g, 14 mmol, 2 equiv.) were refluxed in glacial acetic acid (8 ml) for 5 h. At the end of this period, the precipitated solid was filtered while hot, washed with minimal volume of glacial acetic acid, then with plenty of water and finally with petroleum ether. Drying at 90 °C afforded compounds (**7a-b**), which were used in the next step without further purification.

2-(5-(4-Fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4(5*H*)-one **7a**; white crystals, yield = 75%, m.p. = 209–210 °C (reported m.p. = 210–212 °C).[41]

2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazol-4(5*H*)-one **7b**; white crystals, yield = 75%, m.p. = 216–218 °C (reported m.p. = 220–222 °C).[41]

4.1.5. General preparation of *N*-alkyl isatin derivatives (11a–c). To a stirred DMF solution (6 ml) of isatin (**8a**, 1 g, 6.8 mmol) was portionwise added a DMF solution (2 ml) of the corresponding alkyl halide (7.5 mmol, 1.1 equiv.). Then anhydrous K_2CO_3 (1.9 g, 13.6 mmol, 2 equiv.) and KI (cat.) were added in one portion. The resulting slurry was stirred at 80 °C for 6 h then cooled to room temperature. The reaction mixture was poured portionwise into a beaker containing vigorously stirred cold water/ice mixture (25 ml). The precipitate was allowed to settle down, filtered by suction, air-dried, and then recrystallized from 95% ethanol to afford the desired *N*-alkyl isatin derivatives (**11a–c**).[43]

1-Ethylindoline-2,3-dione **11a**; yellow powder, yield = 84%, m.p. = 85–87 °C.

1-Allylindoline-2,3-dione **11b**; yellow powder, yield = 80%, m.p. = 81–83 °C.

1-Isobutylindoline-2,3-dione **11c**; orange powder, yield = 78%, m.p. = 71–73 °C.

4.1.6. General method for synthesis of 2-(4,5-dihydro-1*H*-pyrazol-1-yl)-5-(isatin-3-ylidene)thiazol-4(5*H*)-one derivatives (9a–h, 10a–h, 12a–c and 13a–c). The corresponding 2-(4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4(5*H*)-one derivative (**7a-b**, 0.5 mmol) as well as the appropriate isatin (**8a–h**) or *N*-alkylisatin (**11a-c**) derivative (0.55 mmol, 1.1 equiv.) and anhydrous sodium acetate (0.12 g, 1.5 mmol, 3 equiv.) were allowed to reflux for 3 h in glacial acetic acid (4 ml). The precipitated solid was subjected to hot filtration, washing with glacial acetic acid, then with plenty of water, and finally with petroleum ether. Recrystallization from ethanol/DMF (4 : 1) followed by drying at 90 °C afforded the desired compounds (**9a–h**, **10a–h**, **12a–c** and **13a–c**) which were sufficiently pure for analysis as illustrated below.

*4.1.6.1. 2-(5-(4-Fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**9a**).* Yellow powder (yield 85%), m.p. >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6), δ 11.14 (s, 1H, –N**H**– 'isatin'), 8.90 (d, J = 7.9 Hz, 1H, Ar-**H**), 7.90–7.82 (m, 2H, Ar-**H**), 7.35 (ddd, J = 13.9, 8.2, 4.4 Hz, 3H, Ar-**H**), 7.22 (td, J = 9.4, 8.7, 2.9 Hz, 2H, Ar-**H**), 7.12–6.99 (m, 3H, Ar-**H**), 6.91 (d, J = 7.7 Hz, 1H, Ar-**H**), 5.95 (dd,

J_{XA} = 11.0, J_{XB} = 4.0 Hz, 1H, pyrazoline-**H**$_X$ (17-C**H**–)), 4.13 (dd, J_{AX} = 18.2, J_{AB} = 11.0 Hz, 1H, pyrazoline-**H**$_A$ (16-C**H**$_A$–)), 3.84 (s, 3H, –OC**H**$_3$), 3.51 (dd, J_{BA} = 18.2, J_{BX} = 4.0 Hz, 1H, pyrazoline-**H**$_B$ (16-C**H**$_B$–)). ^{13}C NMR (101 MHz, DMSO-d_6) δ 179.37 (–**C**=O), 169.48 (–**C**=O), 162.68 (–N–**C**=N), 162.41 (Ar**C**-F), 160.94 (Ar**C**-OCH$_3$), 136.81, 132.15, 130.02, 128.61, 128.48, 126.38, 122.37, 122.29, 122.25, 120.85, 120.21, 120.15, 116.36, 116.14, 116.05, 115.04, 114.99, 110.59, 106.06, 63.49 (–**C**H–), 55.99 (Ar-O**C**H$_3$), 43.84 (–**C**H$_2$–). Elemental analysis for $C_{27}H_{19}FN_4O_3S$: calcd C, 65.05; H, 3.84; N, 11.24, found C, 64.88; H, 3.86; N, 11.31.

*4.1.6.2. 5-(5-Fluoro-2-oxoindolin-3-ylidene)-2-(5-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (**9b**).* Yellow powder (yield 83%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.66 (d, J = 6.6 Hz, 1H, Ar-**H**), 8.19–8.10 (m, 2H, Ar-**H**), 7.62–7.52 (m, 2H, Ar-**H**), 7.45–7.35 (m, 2H, Ar-**H**), 7.39–7.29 (m, 2H, Ar-**H**), 7.33–7.26 (m, 2H, Ar-**H**), 6.32 (dd, J_{XA} = 10.0, J_{XB} = 3.6 Hz, 1H, pyrazoline-**H**$_X$ (17-C**H**–)), 4.57 (dd, J_{AX} = 18.4, J_{AB} = 10.1 Hz, 1H, pyrazoline-**H**$_A$ (16-C**H**$_A$–), 4.17 (s, 3H, –OC**H**$_3$), 3.87 (dd, J_{BA} = 18.3, J_{BX} 3.6 Hz, 1H, pyrazoline-**H**$_B$ (16-C**H**$_B$–)). ^{13}C NMR (101 MHz, TFA-*d*), δ 170.21 (–**C**=O), 170.02 (–**C**=O), 166.11 (–N–**C**=N), 164.29 (Ar**C**-F), 158.68 (Ar**C**-OCH$_3$), 139.24 (Ar**C**-F), 130.90, 129.97, 127.47, 121.85, 120.39, 118.61, 117.26, 116.39, 115.79, 114.88, 112.97, 112.72, 110.15, 66.57 (–**C**H–), 54.96 (Ar-O**C**H$_3$), 45.19 (–**C**H$_2$–). Elemental analysis for $C_{27}H_{18}F_2N_4O_3S$: calcd C, 62.78; H, 3.51; N, 10.85, found C, 62.96; H, 3.49; N, 10.91.

*4.1.6.3. 5-(6-Fluoro-2-oxoindolin-3-ylidene)-2-(5-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (**9c**).* Yellow powder (yield 88%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.80 (s, 1H, Ar-**H**), 8.20 (m, 2H, Ar-**H**), 7.60 (m, 2H, Ar-**H**), 7.50 (t, 8.9 Hz, 1H, Ar-**H**), 7.47–7.29 (m, 4H, Ar-**H**), 6.35 (dd, J = 10.1, 3.6 Hz, 1H, Ar-**H**), 6.35 (dd, J_{XA} = 10.1, J_{XB} = 3.6 Hz, 1H, pyrazoline-**H**$_X$ (17-C**H**–)), 4.61 (dd, J_{AX} = 18.4, J_{AB} = 10.1 Hz, 1H, pyrazoline-**H**$_A$ (16-C**H**$_A$–)), 4.20 (s, 3H, –OC**H**$_3$), 3.91 (dd, J_{BA} = 18.3, J_{BX} 3.6 Hz, 1H, pyrazoline-**H**$_B$ (16-C**H**$_B$–)). ^{13}C NMR (101 MHz, TFA-*d*), δ 170.19 (–**C**=O), 169.50 (–**C**=O), 165.97 (–N–**C**=N), 165.43 (Ar**C**-F), 164.32 (Ar**C**-OCH$_3$), 148.92 (Ar**C**-F), 130.93, 129.99, 127.42, 125.55, 122.17, 121.57, 120.42, 118.66, 117.29, 115.84, 114.91, 113.03, 110.22, 66.59 (–**C**H–), 54.99 (Ar-O**C**H$_3$), 45.23 (–**C**H$_2$–). Elemental analysis for $C_{27}H_{18}F_2N_4O_3S$: calcd C, 62.78; H, 3.51; N, 10.85; found C, 62.93; H, 3.50; N, 10.89.

*4.1.6.4. 5-(6-Chloro-2-oxoindolin-3-ylidene)-2-(5-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (**9d**).* Yellow powder (yield 85%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.89 (s, 1H, Ar-**H**), 8.14 (d, J = 8.6 Hz, 2H, Ar-**H**), 7.64 (dd, J = 8.4, 1.7 Hz, 1H, Ar-**H**), 7.57 (dd, J = 8.6, 4.6 Hz, 2H, Ar-**H**), 7.32 (dt, J = 24.3, 8.4 Hz, 5H, Ar-**H**), 6.33 (dd, J_{XA} = 10.1, J_{XB} = 3.5 Hz, 1H, pyrazoline-**H**$_X$ (17-C**H**–)), 4.57 (dd, J_{AX} = 18.3, J_{AB} = 9.9 Hz, 1H, pyrazoline-**H**$_A$ (16-C**H**$_A$–)), 4.17 (s, 3H, –OC**H**$_3$), 3.87 (dd, J_{BA} = 18.3, J_{BX} 3.3 Hz, 1H, pyrazoline-**H**$_B$ (16-C**H**$_B$–)). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.22 (–**C**=O), 169.82 (–**C**=O), 166.03 (–N–**C**=N), 165.39 (Ar**C**-F), 164.31 (Ar**C**-OCH$_3$), 141.34 (Ar**C**-Cl), 134.98, 130.90, 130.62, 130.00, 129.22, 127.37, 127.00, 120.37, 120.25, 117.27, 115.87, 114.88, 113.05, 110.29, 66.57 (–**C**H–), 54.96 (Ar-O**C**H$_3$), 45.19 (–**C**H$_2$–). Elemental analysis for $C_{27}H_{18}ClFN_4O_3S$: calcd C, 60.85; H, 3.40; N, 10.51, found C, 61.04; H, 3.43; N, 10.46.

*4.1.6.5. 5-(6-Chloro-2-oxoindolin-3-ylidene)-2-(5-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (**9e**).* Yellow powder (yield 80%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.85 (d, J = 6.0 Hz, 1H, Ar-**H**), 8.17 (d, J = 8.2 Hz, 2H, Ar-**H**), 7.68 (d, J = 7.9 Hz, 1H, Ar-**H**), 7.59 (dd, J = 8.7, 4.4 Hz, 2H, Ar-**H**), 7.35 (dd, J = 18.5, 8.0 Hz, 5H, Ar-**H**), 6.37–6.30 (m, 1H, pyrazoline-**H**$_X$ (17-C**H**–)), 4.59 (dd, J_{AX} = 17.5, J_{AB} = 7.7 Hz, 1H, pyrazoline-**H**$_A$ (16-C**H**$_A$–)), 4.19 (s, 3H, –OC**H**$_3$), 3.89 (d, J = 17.9 Hz, 1H, pyrazoline-**H**$_B$ (16-C**H**$_B$–)). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.27 (–**C**=O), 170.21 (–**C**=O), 165.99 (–N–**C**=N), 164.33 (Ar**C**-F), 162.92 (Ar**C**-OCH$_3$), 140.14 (Ar**C**-Cl), 134.79, 130.93, 129.98, 128.75, 128.10, 127.40, 125.40, 120.69, 120.39, 120.39, 117.06, 115.11, 114.90, 66.60 (–**C**H–), 54.97 (Ar-O**C**H$_3$), 45.21 (–**C**H$_2$–). Elemental analysis for $C_{27}H_{18}ClFN_4O_3S$: calcd C, 60.85; H, 3.40; N, 10.51, found 61.03; H, 3.41; N, 10.47.

*4.1.6.6. 2-(5-(4-Fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(5-methoxy-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**9f**).* Red powder (yield 78%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.69 (d, J = 2.5 Hz, 1H, Ar-**H**), 8.13 (d, J = 8.9 Hz, 2H, Ar-**H**), 7.61–7.52 (m, 2H, Ar-**H**), 7.42–7.33 (m, 3H, Ar-**H**), 7.33–7.26 (m, 4H, Ar-**H**), 6.30 (dd, J_{XA} = 10.1, J_{XB} = 3.7 Hz, 1H, pyrazoline-**H**$_X$ (17-C**H**–),4.56 (dd, J_{AX} = 18.4, J_{AB} = 10.1 Hz, 1H, pyrazoline-**H**$_A$ (16-C**H**$_A$–), 4.16 (s, 6H, (–OC**H**$_3$)$_2$, 3.86 (dd, J_{BA} = 18.4, J_{BX} 3.7 Hz, 1H, pyrazoline-**H**$_B$ (16-C**H**$_B$–)). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.09 (–**C**=O), 169.95 (–**C**=O), 166.17 (–N–**C**=N), 165.39 (Ar**C**-OCH$_3$), 164.24 (Ar**C**-F), 162.89 (Ar**C**-OCH$_3$), 155.30, 138.21, 130.87, 127.47, 126.23, 121.75, 120.40, 118.58, 117.26, 116.33, 115.77, 114.86, 112.95, 110.14, 66.52, 56.21 (–**C**H–), 54.95 (Ar-O**C**H$_3$), 45.16 (–**C**H$_2$–). Elemental analysis for $C_{28}H_{21}FN_4O_4S$: calcd C, 63.63; H, 4.00; N, 10.60, found 63.81; H, 3.98; N, 10.66.

*4.1.6.7. 2-(5-(4-Fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(5-methyl-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**9g**).* Yellow powder (yield 75%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.82 (s, 1H, Ar-**H**), 8.30–8.20 (m, 2H, Ar-**H**), 7.73–7.65 (m, 2H, Ar-**H**), 7.63 (d, J = 7.9 Hz, 1H, Ar-**H**), 7.55–7.47 (m, 2H, Ar-**H**), 7.46–7.35 (m, 2H, Ar-**H**), 7.29 (t, J = 6.1 Hz, 1H, Ar-**H**), 6.41 (dd, J_{XA} = 10.2, J_{XB} = 3.8 Hz, 1H, pyrazoline-**H**$_X$ (17-C**H**–), 4.67 (dd, J_{AX} = 18.3, J_{AB} = 10.1 Hz, 1H, pyrazoline-**H**$_A$ (16-C**H**$_A$–), 4.26 (s, 3H, –OC**H**$_3$), 3.97 (dd, J_{BA} = 18.4, J_{BX} 3.7 Hz, 1H, pyrazoline-**H**$_B$ (16-C**H**$_B$–)) 2.64 (s, 3H, –C**H**$_3$). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.21 (–**C**=O), 169.93 (–**C**=O), 166.54 (–N–**C**=N), 164.29 (Ar**C**-F), 163.13 (Ar**C**-OCH$_3$), 141.00, 136.65, 135.58, 130.93, 130.22, 127.60, 124.47, 120.57, 119.49, 118.70, 115.89, 114.97, 113.08, 111.79, 110.26, 66.51 (Ar-O**C**H$_3$), 55.06 (–**C**H–), 45.27 (–**C**H$_2$–), 19.39 (–**C**H$_3$). Elemental analysis for $C_{28}H_{21}FN_4O_3S$: calcd C, 65.61; H, 4.13; N, 10.93, found C, 65.50; H, 4.16; N, 11.00.

*4.1.6.8. 5-(5,7-Dimethyl-2-oxoindolin-3-ylidene)-2-(5-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (**9h**).* Red powder (yield 84%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.52 (d, J = 1.5 Hz, 1H, Ar-**H**), 8.13–8.05 (m, 2H, Ar-**H**), 7.58–7.49 (m, 2H, Ar-**H**), 7.39–7.26 (m, 5H, Ar-**H**), 7.29–7.21 (m, 2H, Ar-**H**), 6.25 (dd, J_{XA} = 10.1, J_{XB} = 3.6 Hz, 1H, pyrazoline-**H**$_X$ (17-C**H**–), 4.51 (dd, J_{AX} = 18.3, J_{AB} = 10.1 Hz,

1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.12 (s, 3H, –OC$\mathbf{H}_3$), 3.81 (dd, J_{BA} = 18.4, J_{BX} 3.7 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–)) 2.46 (s, 3H, –C$\mathbf{H}_3$), 2.39 (s, 3H, –C$\mathbf{H}_3$). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.28 (–**C**=O), 169.69 (–**C**=O), 166.48 (–N–**C**=N), 165.34 (Ar**C**-F), 164.13 (Ar**C**-OCH$_3$), 163.02, 139.26, 138.20, 135.29, 130.76, 130.05, 127.83, 127.43, 124.05, 121.37, 120.44, 116.96, 115.74, 114.81, 112.93, 110.11, 66.32 (Ar-O**C**H$_3$), 54.91 (–**C**H–), 45.11 (–**C**H$_2$–), 19.19 (Ar-**C**H$_3$), 14.03 (Ar-**C**H$_3$). Elemental analysis for $C_{29}H_{23}FN_4O_3S$: calcd C, 66.15; H, 4.40; N, 10.64, found C, 65.96; H, 4.41; N, 10.58.

*4.1.6.9. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**10a**).* Yellow powder (yield 82%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 9.00 (d, J = 8.0 Hz, 1H, Ar-**H**), 8.24 (d, J = 8.0 Hz, 2H, Ar-**H**), 7.80 (t, J = 7.6 Hz, 1H, Ar-**H**), 7.70 (d, J = 8.0 Hz, 2H, Ar-**H**), 7.55–7.34 (m, 6H, Ar-**H**), 6.37 (dd, J_{XA} = 10.2, J_{XB} = 4.0 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.65 (dd, J_{AX} = 17.7, J_{AB} = 9.5 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.26 (s, 3H, –OC$\mathbf{H}_3$), 4.24 (s, 3H, –OC$\mathbf{H}_3$), 3.99 (dd, J_{BA} = 18.4, J_{BX} 4.0 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–)). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.20 (–**C**=O), 170.17 (–**C**=O), 166.42 (–N–**C**=N), 164.27 (Ar**C**-OCH$_3$), 160.17 (Ar**C**-OCH$_3$), 143.16, 135.87, 130.94, 129.88, 127.63, 127.31, 124.86, 120.64, 118.71, 115.90, 114.96, 113.08, 110.27, 66.88 (–**C**H–), 55.23 (Ar-O**C**H$_3$), 55.07 (Ar-O**C**H$_3$), 45.24 (–**C**H$_2$–). Elemental analysis for $C_{28}H_{22}N_4O_4S$: calcd C, 65.87; H, 4.34; N, 10.97, found C, 65.62; H, 4.35; N, 11.05.

*4.1.6.10. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(5-fluoro-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**10b**).* Red powder (yield 87%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.74 (d, J = 6.6 Hz, 1H, Ar-**H**), 8.26–8.19 (m, 2H, Ar-**H**), 7.67–7.60 (m, 2H, Ar-**H**), 7.50–7.36 (m, 6H, Ar-**H**), 6.36 (dd, J_{XA} = 10.0, J_{XB} = 3.8 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.63 (dd, J_{AX} = 18.4, J_{AB} = 10.1 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.25 (s, 3H, –OC$\mathbf{H}_3$), 4.21 (s, 3H, –OC$\mathbf{H}_3$), 3.97 (dd, J_{BA} = 18.4, J_{BX} 3.9 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–)). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.42 (–**C**=O), 170.09 (–**C**=O), 166.06 (–N–**C**=N), 164.31 (Ar**C**-F), 162.56 (Ar**C**-OCH$_3$), 160.14 (Ar**C**-OCH$_3$), 158.76, 139.26, 130.97, 127.53, 127.24, 122.10, 120.55, 118.67, 116.73, 115.92, 115.85, 114.94, 113.04, 110.22, 66.97 (–**C**H–), 55.19 (Ar-O**C**H$_3$), 55.04 (Ar-O**C**H$_3$), 45.22 (–**C**H$_2$–). Elemental analysis for $C_{28}H_{21}FN_4O_4S$: calcd C, 63.63; H, 4.00; N, 10.60, found 63.73; H, 3.98; N, 10.65.

*4.1.6.11. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(7-fluoro-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**10c**).* Red powder (yield 76%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.85 (t, J = 7.2 Hz, 1H, Ar-**H**), 8.31–8.22 (m, 2H, Ar-**H**), 7.69–7.40 (m, 8H, Ar-**H**), 6.40 (dd, J_{XA} = 10.1, J_{XB} = 2.9 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.67 63 (dd, J_{AX} = 10.1, J_{AB} = 18.4 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.28 (s, 3H, –OC$\mathbf{H}_3$), 4.25 (s, 3H, –OC$\mathbf{H}_3$), 4.0 (dd, J_{BA} = 18.4, J_{BX} 3.0 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–)). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.41 (–**C**=O), 169.57 (–**C**=O), 165.95 (–N–**C**=N), 164.34 (Ar**C**-F), 160.18 (Ar**C**-OCH$_3$), 146.55, 131.01, 127.30, 125.46, 122.17, 121.63, 120.59, 118.71, 115.89, 114.97, 113.08, 110.26, 67.00 (–**C**H–), 55.23 (Ar-O**C**H$_3$), 55.08 (Ar-O**C**H$_3$), 45.26 (–**C**H$_2$–). Elemental analysis for $C_{28}H_{21}FN_4O_4S$: calcd C, 63.63; H, 4.00; N, 10.60, found C, 63.81; H, 4.01; N, 10.63.

*4.1.6.12. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(5-chloro-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**10d**).* Orange powder (yield 85%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.99 (s, 1H, Ar-**H**), 8.37–8.22 (m, 2H, Ar-**H**), 7.78–7.65 (m, 3H, Ar-**H**), 7.49–7.35 (m, 5H, Ar-**H**), 6.41 (dd, J_{XA} = 10.1, J_{XB} = 3.9 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.67 (dd, J_{AX} = 17.6, J_{AB} = 9.4 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–) 4.28 (s, 3H, –OC$\mathbf{H}_3$), 4.25 (s, 3H, –OC$\mathbf{H}_3$), 4.01 (dd, J_{BA} = 18.3, J_{BX} 4.2 Hz, 1H, yrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–)). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.48 (–**C**=O), 169.88 (–**C**=O), 165.97 (–N–**C**=N), 164.38 (Ar**C**-OCH$_3$), 160.20 (Ar**C**-OCH$_3$), 141.41, 134.99 (Ar**C**-Cl), 131.01, 130.66, 129.27, 127.59, 127.29, 120.56, 118.72, 115.90, 114.99, 113.09, 110.27, 67.02 (–**C**H–), 55.25 (Ar-O**C**H$_3$), 55.08 (Ar-O**C**H$_3$), 45.25 (–**C**H$_2$–). Elemental analysis for $C_{28}H_{21}ClN_4O_4S$: calcd C, 61.71; H, 3.88; N, 10.28, found C, 61.89; H, 4.00; N, 10.32.

*4.1.6.13. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(7-chloro-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**10e**).* Orange powder (yield 81%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.95 (d, J = 7.1 Hz, 1H, Ar-**H**), 8.30 (d, J = 8.0 Hz, 1H, Ar-**H**), 7.81–7.74 (m, 1H, Ar-**H**), 7.72–7.64 (m, 2H, Ar-**H**), 7.51–7.42 (m, 2H, Ar-**H**), 7.46–7.38 (m, 4H, Ar-**H**), 6.41 (dd, J_{XA} = 10.0, J_{XB} = 3.8 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.67 (dd, J_{AX} = 18.4, J_{AB} = 10.0 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.29 (s, 3H, –OC$\mathbf{H}_3$), 4.26 (s, 3H, –OC$\mathbf{H}_3$), 4.01 (dd, J_{BA} = 18.4, J_{BX} 3.9 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.43 (–**C**=O), 169.68 (–**C**=O), 165.89 (–N–**C**=N), 164.37 (Ar**C**-OCH$_3$), 160.19 (Ar**C**-OCH$_3$), 140.22, 134.78 (Ar**C**-Cl), 131.02, 127.57, 127.31, 125.46, 120.59, 118.71, 117.36, 115.90, 114.98, 113.08, 110.27, 67.02 (–**C**H–), 55.24 (Ar-O**C**H$_3$), 55.09 (Ar-O**C**H$_3$), 45.27 (–**C**H$_2$–). Elemental analysis for $C_{28}H_{21}ClN_4O_4S$: calcd C, 61.71; H, 3.88; N, 10.28, found, 61.83; H, 3.87; N, 10.24.

*4.1.6.14. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(5-methoxy-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**10f**).* Red powder (yield 88%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.84 (d, J = 2.6 Hz, 1H, Ar-**H**), 8.33–8.26 (m, 2H, Ar-**H**), 7.75–7.68 (m, 2H, Ar-**H**), 7.57–7.50 (m, 1H, Ar-**H**), 7.49–7.41 (m, 5H, Ar-**H**), 6.42 41 (dd, J_{XA} = 10.0, J_{XB} = 3.9 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.70 (dd, J_{AX} = 18.4, J_{AB} = 10.1 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), (dd, J = 18.4, 10.1 Hz, 1H), 4.32 (s, 3H, –OC$\mathbf{H}_3$), 4.31 (s, 3H, –OC$\mathbf{H}_3$), 4.28 (s, 3H, –OC$\mathbf{H}_3$), 4.04 (dd, J_{BA} = 18.4, J_{BX} 3.9 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–)). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.38 (–**C**=O), 170.12 (–**C**=O), 166.20 (–N–**C**=N), 164.35 (Ar**C**-OCH$_3$), 160.22 (Ar**C**-OCH$_3$), 155.45 (Ar**C**-OCH$_3$), 138.31, 131.01, 127.59, 127.34, 121.87, 120.65, 118.74, 116.41, 116.01, 115.93, 115.01, 113.11, 112.89, 110.30, 67.01 (–**C**H–), 56.36 (Ar-O**C**H$_3$), 55.26 (Ar-O**C**H$_3$), 55.11 (Ar-O**C**H$_3$), 45.26 (–**C**H$_2$–). Elemental analysis for $C_{29}H_{24}N_4O_5S$: calcd C, 64.43; H, 4.48; N, 10.36, found C, 64.27; H, 4.51; N, 10.39.

*4.1.6.15. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(5-methyl-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**10g**).* Red powder (yield 80%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.87 (s, 1H, Ar-**H**), 8.28 (dd, J = 4.3, 2.3 Hz, 2H, Ar-**H**), 7.79–7.72 (m, 2H, Ar-**H**), 7.72–7.64 (m, 1H, Ar-**H**), 7.55–7.38 (m, 5H, Ar-**H**), 6.43 (dd, J_{XA} = 9.9, J_{XB} = 3.7 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.70 (dd, J_{AX} = 18.4, J_{AB} = 10.0 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.30 (s, 3H, –OC$\mathbf{H}_3$), 4.30 (s, 3H, –OC$\mathbf{H}_3$), 4.04 (dd, J_{BA} =

18.4, J_{BX} 3.7 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–)) 2.69 (s, 3H, –C$\mathbf{H}_3$). ^{13}C NMR (101 MHz, TFA-*d*) δ 170.29 (–**C**=O), 170.12 (–**C**=O), 166.44 (–N–**C**=N), 164.30 (Ar**C**-OCH$_3$), 162.92 (Ar**C**-OCH$_3$), 160.23, 140.98, 136.62, 130.97, 130.26, 127.73, 127.36, 124.61, 120.69, 118.77, 115.95, 115.01, 113.14, 111.81, 110.33, 66.89 (–**C**H–), 55.29 (Ar-O**C**H$_3$), 55.10 (Ar-O**C**H$_3$), 45.26 (–**C**H$_2$–), 19.46 (Ar-**C**H$_3$). Elemental analysis for $C_{29}H_{24}N_4O_4S$: calcd C, 66.40; H, 4.61; N, 10.68 found C, 66.58; H, 4.59; N, 10.73.

*4.1.6.16. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(5,7-dimethyl-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**10h**).* Red powder (yield 84%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.93 (s, 1H, Ar-**H**), 8.49 (d, J = 5.3 Hz, 2H, Ar-**H**), Ar-**H**, 8.04–7.99 (m, 2H, Ar-**H**), 7.75 (s, 3H, Ar-**H**), 7.73 (d, J = 5.1 Hz, 2H, Ar-**H**), 7.67–7.60 (m, 2H, Ar-**H**), 6.68 (s, 1H, pyrazoline-**H**), 4.96–4.85 (m, 1H, pyrazoline-**H**), 4.61–4.51 (m, 6H, (–O**C**H$_3$)$_2$), 4.31–4.26 (m, 1H, pyrazoline-**H**), 2.90 (s, 3H, –C$\mathbf{H}_3$), 2.80 (s, 3H, –C$\mathbf{H}_3$). ^{13}C NMR (101 MHz, TFA-*d*) δ 173.88 (–**C**=O), 173.64 (–**C**=O), 170.25 (–N–**C**=N), 164.60 (Ar**C**-OCH$_3$), 147.75 (Ar**C**-OCH$_3$), 138.53, 131.21, 128.23, 127.94, 127.63, 119.07, 116.24, 115.25, 113.44, 110.61, 67.13 (–**C**H–), 55.59 (Ar-O**C**H$_3$), 55.41 (Ar-O**C**H$_3$), 45.54 (–**C**H$_2$–), 19.78 (Ar-**C**H$_3$), 14.63 (Ar-**C**H$_3$). Elemental analysis for $C_{30}H_{26}N_4O_4S$: calcd C, 66.90; H, 4.87; N, 10.40, found C, 67.11; H, 4.85; N, 10.46.

*4.1.6.17. 5-(1-Ethyl-2-oxoindolin-3-ylidene)-2-(5-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (**12a**).* Orange powder (yield 73%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.84 (d, J = 7.9 Hz, 1H, Ar-**H**), 8.11–8.05 (m, 2H, Ar-**H**), 7.66 (t, J = 7.6 Hz, 1H, Ar-**H**), 7.50 (dd, J = 8.6, 4.7 Hz, 2H, Ar-**H**), 7.33 (t, J = 7.8 Hz, 1H, Ar-**H**), 7.31–7.20 (m, 4H, Ar-**H**), 7.16 (d, J = 7.9 Hz, 1H, Ar-**H**), 6.21 (dd, J_{XA} = 10.1, J_{XB} = 3.8 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.51 (dd, J_{AX} = 18.3, J_{AB} = 10.1 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.10 (s, 3H, –OC$\mathbf{H}_3$), 4.18–4.16 (m, 2H, –C$\mathbf{H}_2$), 3.79 (dd, J_{BA} = 18.4, J_{BX} 3.7 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–), 1.59 (t, J = 7.0 Hz, 3H, –C$\mathbf{H}_3$). ^{13}C NMR (101 MHz, TFA-*d*) δ 168.77 (–**C**=O), 167.91 (–**C**=O), 165.99 (–N–**C**=N), 164.00 (Ar**C**-OCH$_3$), 162.00 (Ar**C**-F), 144.10, 135.14, 133.21 130.17, 129.19, 127.31, 127.09, 124.11, 120.17, 119.10, 115.10, 114.16, 109.24, 66.13 (–**C**H–), 55.20 (Ar-O**C**H$_3$), 54.27 (Ar-O**C**H$_3$), 45.03 (–**C**H$_2$–), 35.23 (–**C**H$_2$–), 10.13 (–**C**H$_3$). Elemental analysis for $C_{29}H_{23}FN_4O_3S$: calcd C, 66.15; H, 4.40; N, 10.64, found C, 65.93; H, 4.41; N, 10.71.

*4.1.6.18. 5-(1-Allyl-2-oxoindolin-3-ylidene)-2-(5-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (**12b**).* Orange powder (yield 77%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.87 (dd, J = 8.0, 1.1 Hz, 1H, Ar-**H**), 8.14–8.05 (m, 2H, Ar-**H**), 7.66 (td, J = 7.8, 1.1 Hz, 1H, Ar-**H**), 7.55–7.46 (m, 2H, Ar-**H**), 7.35 (td, J = 7.8, 0.9 Hz, 1H), 7.33–7.23 (m, 4H, Ar-**H**), 7.17 (d, J = 7.9 Hz, 1H, Ar-**H**), 6.22 (dd, J_{XA} = 10.1, J_{XB} = 3.7 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 6.01 (ddt, J = 17.1, 10.4, 5.2 Hz, 1H, –C**H**=), 5.50–5.38 (m, 2H, =C$\mathbf{H}_2$), 4.64 (dt, J = 5.4, 1.6 Hz, 2H, –C$\mathbf{H}_2$–), 4.50 (dd, J_{AX} = 18.3, J_{AB} = 10.1 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.11 (s, 3H), 3.80 (dd, J_{BA} = 18.4, J_{BX} 3.7 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–). ^{13}C NMR (101 MHz, TFA-*d*) δ 169.77 (–**C**=O), 167.96 (–**C**=O), 166.46 (–N–**C**=N), 164.13 (Ar**C**-OCH$_3$), 162.93 (Ar**C**-F), 144.70, 135.42, 133.62, 130.81, 129.74, 128.44, 127.45, 124.82, 120.41, 118.98, 118.05, 116.94, 115.71, 114.81, 112.89, 110.62 (=**C**H–), 110.08 (=**C**H$_2$–), 66.37 (–**C**H–), 54.90 (Ar-O**C**H$_3$), 45.13 (–**C**H$_2$–), 43.04 (–**C**H$_2$–). Elemental analysis for $C_{30}H_{23}FN_4O_3S$: calcd C, 66.90; H, 4.30; N, 10.40, found C, 67.13; H, 4.31; N, 10.36.

*4.1.6.19. 2-(5-(4-Fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(1-isobutyl-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**12c**).* Yellow powder (yield 73%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.87 (d, J = 7.9 Hz, 1H, Ar-**H**), 8.15–8.07 (m, 2H, Ar-**H**), 7.68 (t, J = 7.6 Hz, 1H, Ar-**H**), 7.52 (dd, J = 8.6, 4.7 Hz, 2H, Ar-**H**), 7.35 (t, J = 7.8 Hz, 1H, Ar-**H**), 7.33–7.22 (m, 4H, Ar-**H**), 7.18 (d, J = 7.9 Hz, 1H, Ar-**H**), 6.23 (dd, J_{XA} = 10.1, J_{XB} = 3.7 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.50 (dd, J_{AX} = 18.3, J_{AB} = 10.1 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.13 (s, 3H, –OC$\mathbf{H}_3$), 3.84 (d, J = 7.7 Hz, 2H, –C$\mathbf{H}_2$–), 3.78 (dd, J_{BA} = 18.4, J_{BX} 3.7 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–), 2.42–2.30 (m, 1H, (–C**H**–), 1.17 (dd, J = 6.7, 1.5 Hz, 6H, (–C$\mathbf{H}_3$)$_2$). ^{13}C NMR (101 MHz, TFA-*d*) δ 169.73 (–**C**=O), 168.21 (–**C**=O), 166.56 (–N–**C**=N), 164.13 (Ar**C**-OCH$_3$), 163.03 (Ar**C**-F), 145.26, 135.39, 133.77, 130.82, 129.98, 129.79, 127.45, 124.74, 120.43, 118.98, 117.17, 115.78, 114.82, 112.97, 110.41, 66.35 (–**C**H$_2$–), 54.91 (Ar-O**C**H$_3$), 48.51 (–**C**H$_2$–), 45.13 (–**C**H$_2$–), 27.00 (–**C**H–), 18.34 (–C$\mathbf{H}_3$)$_2$). Elemental analysis for $C_{31}H_{27}FN_4O_3S$: calcd C, 67.13; H, 4.91; N, 10.10, found C, 67.29; H, 4.88; N, 10.16.

*4.1.6.20. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(1-ethyl-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**13a**).* Orange powder (yield 80%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.91 (d, J = 7.8 Hz, 1H, Ar-**H**), 8.15 (d, J = 8.5 Hz, 2H, Ar-**H**), 7.74 (t, J = 7.7 Hz, 1H, Ar-**H**), 7.55 (d, J = 8.3 Hz, 2H, Ar-**H**), 7.40 (t, J = 7.7 Hz, 1H, Ar-**H**), 7.33–7.26 (m, 4H, Ar-**H**), 7.24 (d, J = 7.9 Hz, 1H, Ar-**H**), 6.24 43 (dd, J_{XA} = 10.0, J_{XB} = 3.6 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.53 (dd, J_{AX} = 18.3, J_{AB} = 9.8 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.18 (s, 3H, –OC$\mathbf{H}_3$), 4.16–4.14 (m, 2H, –C$\mathbf{H}_2$–), 4.12 (s, 3H, –OC$\mathbf{H}_3$), 3.87 (dd, J_{BA} = 18.3, J_{BX} 3.3 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–), 1.58 (t, J = 7.0 Hz, 3H, –C$\mathbf{H}_3$). ^{13}C NMR (101 MHz, TFA-*d*) δ 169.93 (–**C**=O), 167.77 (–**C**=O), 166.50 (–N–**C**=N), 164.12 (Ar**C**-OCH$_3$), 160.00 (Ar**C**-OCH$_3$), 144.50, 135.45, 133.84, 130.85, 129.91, 127.51, 127.19, 124.81, 120.57, 119.20, 115.80, 114.86, 109.94, 66.73 (–**C**H–), 55.10 (Ar-O**C**H$_3$), 54.97 (Ar-O**C**H$_3$), 45.13 (–**C**H$_2$–), 35.83 (–**C**H$_2$–), 10.83 (–**C**H$_3$). Elemental analysis for $C_{30}H_{26}N_4O_4S$: calcd C, 66.90; H, 4.87; N, 10.40, found C, 67.08; H, 4.88; N, 10.37.

*4.1.6.21. 5-(1-Allyl-2-oxoindolin-3-ylidene)-2-(3,5-bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (**13b**).* Yellow powder (yield 73%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.94 (d, J = 7.5 Hz, 1H, Ar-**H**), 8.17 (d, J = 8.2 Hz, 2H, Ar-**H**), 7.74 (t, J = 7.6 Hz, 1H, Ar-**H**), 7.57 (d, J = 7.9 Hz, 2H, Ar-**H**), 7.42 (t, J = 7.2 Hz, 1H, Ar-**H**), 7.31 (dd, J = 8.3, 4.8 Hz, 4H, Ar-**H**), 7.24 (d, J = 7.8 Hz, 1H, Ar-**H**), 6.26 (dd, J_{XA} = 8.3, J_{XB} = 3.5 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 6.08 (ddt, J = 13.8, 7.3 Hz, 1H, –C**H**=), 5.57–5.45 (m, 2H, =C$\mathbf{H}_2$), 4.72 (d, J = 4.7 Hz, 2H, –C$\mathbf{H}_2$–), 4.55 (dd, J_{AX} = 17.9, J_{AB} = 8.3 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.19 (s, 3H, –OC$\mathbf{H}_3$), 4.14 (s, 3H, –OC$\mathbf{H}_3$), 3.89 (dd, J_{BA} = 17.9, J_{BX} 3.5 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–). ^{13}C NMR (101 MHz, TFA-*d*) δ 169.99 (–**C**=O), 168.10 (–**C**=O), 166.48 (–N–**C**=N), 164.16 (Ar**C**-OCH$_3$), 160.03 (Ar**C**-OCH$_3$), 144.73, 135.44, 133.61, 130.87, 129.79, 128.51, 127.50, 127.21, 124.89, 120.56, 119.07, 118.10 (=**C**H$_2$–), 115.83, 114.87, 110.69, 66.77 (–**C**H–), 55.12 (Ar-O**C**H$_3$), 54.98 (Ar-O**C**H$_3$), 45.15 (–**C**H$_2$–), 43.11 (–**C**H$_2$–).

Elemental analysis for $C_{31}H_{26}N_4O_4S$: calcd C, 67.62; H, 4.76; N, 10.18, found 67.83; H, 4.74; N, 10.11.

*4.1.6.22. 2-(3,5-Bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-(1-isobutyl-2-oxoindolin-3-ylidene)thiazol-4(5H)-one (**13c**).* Yellow powder (yield 72%), m.p. >300 °C, ^{1}H NMR (400 MHz, TFA-*d*), δ 8.86 (d, J = 7.9 Hz, 1H, Ar-**H**), 8.13–8.06 (m, 2H, Ar-**H**), 7.67 (t, J = 7.7 Hz, 1H, Ar-**H**), 7.49 (d, J = 8.6 Hz, 2H, Ar-**H**), 7.33 (t, J = 7.8 Hz, 1H, Ar-**H**), 7.23 (t, J = 8.9 Hz, 4H, Ar-**H**), 7.16 (d, J = 8.0 Hz, 1H, Ar-**H**), 6.18 (dd, J_{XA} = 10.1, J_{XB} = 3.8 Hz, 1H, pyrazoline-$\mathbf{H}_X$ (17-C**H**–), 4.46 (dd, J_{AX} = 18.4, J_{AB} = 10.1 Hz, 1H, pyrazoline-$\mathbf{H}_A$ (16-C$\mathbf{H}_A$–), 4.09 (d, J = 22.3 Hz, 6H, (–OC$\mathbf{H}_3$)$_2$), 3.83 (d, J = 7.1 Hz, 2H, –C$\mathbf{H}_2$–), 3.78 (dd, J_{BA} = 18.4, J_{BX} 3.8 Hz, 1H, pyrazoline-$\mathbf{H}_B$ (16-C$\mathbf{H}_B$–), 2.35 (dt, J = 13.7, 6.9 Hz, 1H, –C**H**–), 1.16 (dd, J = 6.6, 2.0 Hz, 6H, (–C$\mathbf{H}_3$)$_2$). ^{13}C NMR (101 MHz, TFA-*d*) δ 169.84 (–**C**=O), 168.21 (–**C**=O), 166.43 (–N–**C**=N), 164.05 (Ar**C**-OCH$_3$), 159.92 (Ar**C**-OCH$_3$), 145.18, 135.29, 133.58, 130.79, 129.74, 127.47, 127.13, 124.85, 124.71, 120.52, 118.97, 118.54, 115.73, 114.79, 112.91, 110.38, 110.10, 66.65 (–**C**H$_2$–), 55.03 (Ar-O**C**H$_3$), 54.90 (Ar-O**C**H$_3$), 48.49 (–**C**H$_2$–), 45.06 (–**C**H$_2$–), 26.99 (–**C**H–), 18.33 (–C**H**$_3$)$_2$). Elemental analysis for $C_{32}H_{30}N_4O_4S$: calcd C, 67.83; H, 5.34; N, 9.89, found C, 68.07; H, 5.31; N, 9.83.

4.2. Biological evaluation

The experimental procedures for antimicrobial XTT[44,45] and SRB cytotoxicity[37,38] assays are provided in the ESI.†

Conflicts of interest

No potential conflict of interest was reported by the author(s).

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