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Short communication

Synthesis and antimicrobial activity of new 1-alkyl/cyclohexyl-3,3-diaryl-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-diones

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Abstract

The paper describes the synthesis of new 1-alkyl/cyclohexyl-3,3-diaryl-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-diones from the reactions of the 2-diazo-1,2-diarylethanones with 1-methyl-3-(alkyl/cyclohexylimino)indolin-2-ones under thermal condition. The compounds, characterized by satisfactory analytical and spectral (IR, ^{1}H NMR and ^{13}C NMR) data, have been screened for their antibacterial and antifungal activities.

Keywords: Spiroazetidinones; Diarylketenes; 3-Iminoindolin-2-ones; Cycloaddition; Antimicrobial activity

1. Introduction

The 2-azetidinones, commonly known as β-lactams, are among the most useful azaheterocyclic compounds from both synthetic and medicinal chemistry points of views. Most of the researches up to early 90s focused on synthesis of 2-azetidinones and study of their antibacterial property [1–3]. Later researches have shown some other useful biological activities of such compounds, e.g. cholesterol absorption inhibition, enzymes inhibition, hypoglycemic, and anticancer activity [4,5]. Ezetimibe, a monocyclic 2-azetidinone, is now in clinical use as a cholesterol absorption inhibitor [6]. Recent years have shown extensive investigation on use of 2-azetidinones as synthons for diverse types of biologically important compounds such as β-aminoacids, γ-aminoalcohols, azetidines, pyrimidones, etc. [7–11]. However, most of these studies are undertaken either on monocyclic or fused bicyclic 2-azetidinones. It is due to the fact that the first 2-azetidinone-containing antibiotics penicillin and cephalosporin had fused bicyclic skeletons whereas monobactams and nocardicins, observed in late 70s and early 80s to have antibacterial activity, had monocyclic 2-azetidinone ring. The spiro-fused 2-azetidinones constitute a scarce class of compounds. There are only a few reports in the literature on the synthesis, reactivity and biological activity of such 2-azetidinones [12–27]. The diverse types of biological activities associated with indoline-2,3-dione (isatin) derivatives and their interesting chemistry [28–31] prompted us to synthesize 2-azetidinones spiro-fused to 1-methylindolin-2-one ring and study its antimicrobial activity. Accordingly, the present paper reports the synthesis and antimicrobial activity of 10 new 1-alkyl/cyclohexyl-3,3-diaryl-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-diones from the reactions of 1-methyl-3-(alkyl/cyclohexylimino)indolin-2-ones with three 2-diazo-1,2-diarylethanones – 2-diazo-1,2-diphenylethanone, 2-diazo-1,2-bis(4-methylphenyl)ethanone and 2-diazo-1,2-bis(4-methoxyphenyl)ethanone.

There are several methods reported in the literature for the synthesis of 2-azetidinones [9,10]. However, the Staudinger ketene–imine cycloaddition reaction is one of the most common methods to synthesize 2-azetidinones [32]. It involves reaction of an imine with acid chloride in the presence of a tertiary base, usually triethylamine at temperatures ranging from −78 °C to reflux temperature in various solvents such as dichloromethane, acetonitrile, toluene, etc. (Scheme 1). In the present study, diarylketenes are generated *in situ* by thermal decomposition of α-diazoketones because their reactions appeared versatile, simpler to carry out requiring

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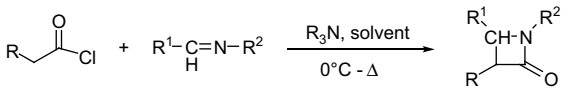


Scheme 1. The Staudinger method for the preparation of 2-azetidinones.

no temperature control, no acid or base treatment and also no water work-up.

2. Results and discussion

2.1. Chemistry

An equimolar reaction of 2-diazo-1,2-diphenylethanone (**1a**) with 1-methyl-3-(isopropylimino)indolin-2-one (**2a**) in dry benzene afforded a white crystalline compound characterized as 1-isopropyl-1′-methyl-3,3-diphenylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3a**) on the basis of satisfactory analytical and spectral data (see Section 4).

Similar reactions of 2-diazo-1,2-diphenylethanone (**1a**) with 1-methyl-3-(*N*-substituted imino)indolin-2-ones (**2b–d**) also afforded spiro(azetidin-2,3′-indoline)-2′,4-diones (**3b–d**) in good yields (Table 1). The reactions of 2-diazo-1,2-bis(4-methylphenyl)ethanone (**1b**) and of 2-diazo-1,2-bis(4-methoxyphenyl)ethanone (**1c**) were carried out in a similar manner with 1-methyl-3-(alkylimino)indolin-2-ones **2a–d** and with **2a** and **b**, respectively, which yielded new spiro(azetidin-2,3′-indoline)-2′,4-diones (**3e–h**) and (**3i,j**), respectively, in good to excellent yields (Table 1) which have been characterized on the basis of satisfactory analytical and spectral data discussed briefly in the succeeding paragraph.

The IR spectra of compounds **3a–j** showed two strong absorption bands – one at around 1745–1760 and the other at around 1720–1730 cm^{-1} corresponding to two lactam carbonyl groups. The ^{13}C NMR spectra showed the two carbonyl carbons at around δ 173 and 169 ppm. In the IR and ^{13}C spectra of the products **3a–j**, the disappearance of the band at around 1640 cm^{-1} corresponding to azomethine linkage and of signal at around δ 155 ppm corresponding to azomethine carbon, respectively, in substrates **2a–d** confirmed the reaction at azomethine linkage of the substrates forming spiro[azetidine-2,3′-indoline]-2′,4-diones. It is noteworthy to mention that the ^1H NMR spectra of spiro-compounds **3c** and **g** with α-phenylmethyl group on azetidinone ring nitrogen showed the signals in sets of two (approximately in the ratio of 9:1) indicating two spatial arrangement of the groups in these compounds. The *N*-methyl and methine signals for minor configuration appeared slightly downfield than the signals for these protons in major configurations whereas the signal for methyl proton [CH(Me)Ph] appeared slightly upfield. The mass spectra of the products recorded showed the quasimolecular ions formed by addition of sodium $(M + Na)^+$.

Table 1
Physical data of the spiro[azetidin-2,3′-indoline]-2′,4-diones **3a–j**

Compound number	Ar	R	Molecular formula	M.p. (°C)	Yield (%)
3a	Ph	$CHMe_2$	$C_{26}H_{24}N_2O_2$	200–202	69
3b	Ph	$CHPh_2$	$C_{36}H_{28}N_2O_2$	236–237	74
3c	Ph	CH(Me)Ph	$C_{31}H_{26}N_2O_2$	162–165	60
3d	Ph	Cyclohexyl	$C_{29}H_{28}N_2O_2$	155–158	50
3e	4-MeC_6H_4	$CHMe_2$	$C_{28}H_{28}N_2O_2$	258–260	72
3f	4-MeC_6H_4	$CHPh_2$	$C_{38}H_{32}N_2O_2$	194–195	78
3g	4-MeC_6H_4	CH(Me)Ph	$C_{33}H_{30}N_2O_2$	214–216	68
3h	4-MeC_6H_4	Cyclohexyl	$C_{31}H_{32}N_2O_2$	180–182	52
3i	4-$MeOC_6H_4$	$CHMe_2$	$C_{28}H_{28}N_2O_4$	255–258	77
3j	4-$MeOC_6H_4$	$CHPh_2$	$C_{38}H_{32}N_2O_4$	204–206	80

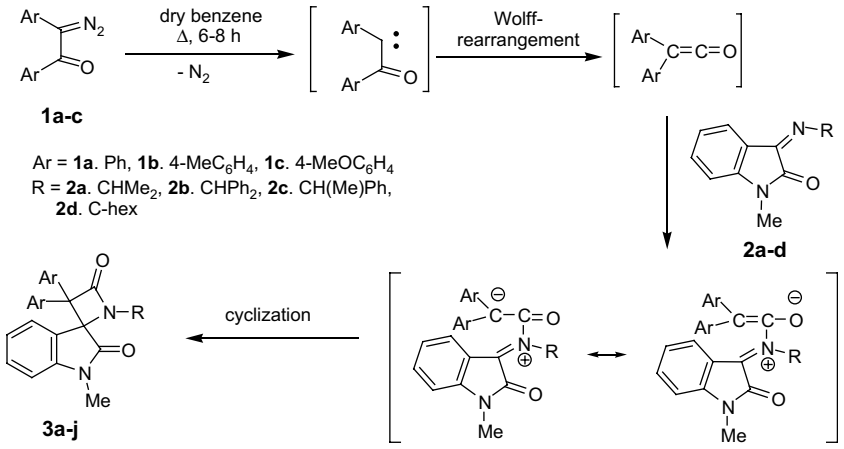
The mechanism of formation of the product, which is similar to the one proposed earlier for such reactions [28], is shown in Scheme 2. Thermal decomposition of the 2-diazoketones leads to the formation of α-ketocarbenes with extrusion of nitrogen. The α-ketocarbenes are known to undergo the Wolff rearrangement leading to *in situ* generation of ketenes [33]. The reaction of imines with ketenes may lead to the formation of a *zwitterionic* intermediate, which cyclizes to give the product 2-azetidinones.

2.2. Pharmacology

All the synthesized compounds were screened for their antibacterial and antifungal activities (Table 2). The concentrations ranging from 1.0 to 100.0 μg mL^{-1} of the test compound were used for the screening. The bacterial strains used were Gram-(+) *Bacillus subtilis*, *Staphylococcus aureus* and Gram-(−) *Escherichia coli* and *Pseudomonas aeruginosa*. The antifungal screening was done on *Candida albicans* and on *Saccharomyces cerevisiae*. Of the 10 compounds screened against bacterial strains, four compounds showed activity on *E. coli* (**3d**: MIC = 100 μg mL^{-1}, **3e**: MIC = 10 μg mL^{-1}, **3h**: MIC = 100 μg mL^{-1}, **3j**: MIC = 50 μg mL^{-1}) whereas only one compound showed activity on *P. aeruginosa* (**3e**: MIC = 50 μg mL^{-1}). None of the compounds showed activity on Gram-(+) strains up to the maximum concentration of 100 μg mL^{-1} used in the study. These results are in agreement with previous studies on monocyclic 2-azetidinones which reported higher activity of such 2-azetidinones against Gram-(−) bacteria in comparison to that against Gram-(+) bacteria [34–36]. Although none of the compounds showed activity up to 100 μg mL^{-1} against *C. albicans* six compounds (**3a**: MIC = 50 μg mL^{-1}, **3b**. MIC = 10 μg mL^{-1}, **3e**: MIC = μg mL^{-1}, 50, **3f**: MIC = 100 μg mL^{-1}, **3i**: MIC = 50 μg mL^{-1}, **3j**: MIC = 50 μg mL^{-1}) have been observed active on *S. cerevisiae*. Only compound **3e** with two 4-methylphenyl groups and an isopropyl group on 2-azetidinone ring carbon and nitrogen, respectively, showed activity against three organisms out of five studied.

3. Conclusions

In conclusion, the paper reports the synthesis of spiro-compounds containing 2-azetidinone and 1-methylindolin-2-one rings from the reactions of 1-methyl-3-(alkylimino) indolin-2-ones with three diarylketenes – diphenylketene,



Scheme 2. Mechanism of formation of compounds **3a–j**.

bis(4-methylphenyl)ketene and bis(4-methoxyphenyl)ketene. Some of the compounds selectively inhibited the microbes.

4. Experimental

4.1. Chemistry

Melting points have been recorded on a Stuart Scientific melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin–Elmer-781 IR spectrophotometer using KBr disc of the sample. The ^{1}H and ^{13}C NMR spectra were recorded in a $CDCl_3$ solution at 300 and 75.4 MHz, respectively, on a Bruker™ spectrometer. The mass spectra were recorded on Finnigan LC Q^{DECA} mass spectrometer by electrospray ionization. The elemental analyses were performed on Gmbh VarioEL elemental analyzer.

Benzil, 4,4-dimethylbenzil, 4,4-dimethoxylbenzil, 1-methylindolin-2,3-dione, isopropyl amine, diphenylmethanamine, (±)-α-phenylethyl amine, cyclohexyl amine and hydrazine hydrate were Sigma products. The benzene was dried by refluxing over NaH. 2-Diazo-1,2-diarylethanones **1** were prepared by oxidation of appropriate benzil monohydrazones using yellow mercuric oxide according to the reported method [37].

Table 2
Antimicrobial activity of the compounds (MIC, μg mL^{-1})[a]

Compound number	Ar	R	*E. coli*	*P. aeruginosa*	*S. cerevisiae*
3a	Ph	$CHMe_2$			50
3b	Ph	$CHPh_2$			10
3d	Ph	Cyclohexyl	100		
3e	4-MeC_6H_4	$CHMe_2$	10	50	50
3f	4-MeC_6H_4	$CHPh_2$			100
3h	4-MeC_6H_4	Cyclohexyl	100		
3i	4-$MeOC_6H_4$	$CHMe_2$			50
3j	4-$MeOC_6H_4$	$CHPh_2$	50		50
Chloramphenicol			0.1	0.1	
Miconazole					0.1

[a] None of the compounds except reference compound chloramphenicol (MIC = 0.1 μg mL^{-1}) showed activity against *B. subtilis* and *S. aureus* whereas miconazole showed activity against *C. albicans* (MIC = 0.1 μg mL^{-1}).

Microbial cultures were developed in the Department of Biological Sciences at the University of Botswana.

*4.1.1. Preparation of 1-methyl 3-(alkylimino)indolin-2-ones (**2a–d**)*

An equimolar amount (10 mmol) of an appropriate amine and 1-methylindolin-2,3-dione in ethanol was refluxed for 4–6 h. The reaction mixture was kept overnight at room temperature to afford red crystalline products which were used as such for the reaction.

*4.1.2. Synthesis of 1-alkyl/cyclohexyl-3,3-diaryl-1′-methylspiro [azetidine-2,3′-indoline]-2′,4-diones (**3a–j**)*

An equimolar amount of 2-diazo-1,2-diarylethanones **1** and iminoindolin-2-ones **2** (1 mmol of each) in 10 mL of dry benzene was heated to reflux under an atmosphere of nitrogen for 6–8 h. The solvent was evaporated under reduced pressure using a rotary evaporator. The residue was triturated with ethanol to afford the white crystalline products **3** which were checked for purity by TLC using *n*-hexane: ethyl acetate (1:1) as eluent. The analytical and spectral data are given below.

*4.1.2.1. 1-Isopropyl-3,3-diphenyl-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3a**).* (IR, KBr, cm^{-1}): 1745, 1720; ^{1}H NMR ($CDCl_3$, δ ppm): 7.50–6.19 (14H, arom.), 3.70 (sept, 1H, methine), 3.27 (s, 3H, N–CH_3), 1.25 (d, $J = 6.0$ Hz, 3H, CH_3), 1.11 (d, $J = 6.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): 174.7, 168.3, 143.8, 138.5, 130.2, 128.2, 128.1, 127.3, 127.2, 126.8, 126.8, 124.2, 122.0, 108.3, 75.8, 71.3, 46.7, 26.7, 21.7, 20.6; Mass (*m/z*, r.i.): 419 $(M + Na, 13)^+$, 416 (20), 388 (15), 312 (16), 225 $[(M + Na) - (Ph_2C{=}C{=}O), 100]^+$; Elemental analysis: Calcd for $C_{26}H_{24}N_2O_2$: C 78.76, H 6.10, N 7.07%; Found: C 78.22, H 5.94, N 6.89%.

*4.1.2.2. 1-Diphenylmethyl-3,3-diphenyl-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3b**).* (IR, KBr, cm^{-1}): 1760, 1728; ^{1}H NMR ($CDCl_3$, δ ppm): 7.47–6.07 (24H, arom.), 5.88 (s, 1H, methine), 2.88 (s, 3H, N–CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): 174.0, 169.5, 143.9, 138.8, 136.2, 130.0,

129.9, 128.3, 128.2, 127.7, 127.6, 127.5, 127.5, 127.4, 127.2, 127.1, 126.6, 123.9, 121.6, 107.9, 75.3, 71.4, 62.8, 26.3; Mass (*m/z*, r.i): 544 $(M + Na, 35)^+$, 543 (100); Elemental analysis: Calcd for $C_{36}H_{28}N_2O_2$: C 83.05, H 5.42, N 5.38%; Found: C 82.1, H 5.30, N 5.30%.

*4.1.2.3. 1-(α-Phenylethyl)-3,3-diphenyl-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3c**).* (IR, KBr, cm^{-1}): 1753, 1720; ^1H NMR ($CDCl_3$, δ ppm): 7.46–5.93 (19H, arom.), 4.69 (q, $J = 6.0$ Hz, 1H, methine), 2.96 (s, 3H, N–CH_3), 1.76 (d, $J = 6.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): 173.9, 169.0, 143.9, 138.6, 138.5, 138.0, 129.9, 128.3, 128.2, 128.2, 128.1, 128.0, 127.7, 127.4, 127.1, 127.0, 126.7, 123.7, 121.6, 107.9, 75.5, 71.3, 55.2, 26.4, 20.6; Mass (*m/z*, r.i.): 482 $(M + Na, 32)^+$, 481 (100); Elemental analysis: Calcd for $C_{31}H_{26}N_2O_2$: C 81.20, H 5.72, N 6.11%; Found: C 81.0, H 5.43, N 5.94%.

*4.1.2.4. 1-Cyclohexyl-3,3-diphenyl-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3d**).* (IR, KBr, cm^{-1}): 1750, 1721; ^1H NMR ($CDCl_3$, δ ppm): 7.49–6.18 (14H, arom.), 3.37 (tt, 1H, N–CH), 3.27 (s, 3H, N–CH_3), 2.05–1.05 (m, 10H, C-hex); ^{13}C NMR ($CDCl_3$, δ ppm): 174.7, 168.3, 143.8, 138.5, 130.2, 128.2, 128.2, 127.3, 127.2, 126.9, 126.8, 124.2, 122.0, 108.3, 75.8, 71.3, 54.3, 31.8, 30.7, 26.7, 25.2, 25.1; Elemental analysis: Calcd for $C_{29}H_{28}N_2O_2$: C 79.79, H 6.46, N 6.42%; Found: C 79.39, H 6.26, N 6.26%.

*4.1.2.5. 1-Isopropyl-3,3-bis(4-methylphenyl)-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3e**).* (IR, KBr, cm^{-1}): 1748, 1720; ^1H NMR ($CDCl_3$, δ ppm): 7.31–6.25 (12H, arom.), 3.71 (sept, $J = 6.0$ Hz, 1H, methine), 3.26 (s, 3H, N–CH_3), 2.27 and 2.26 (2s, 6H, Ph-CH_3), 1.25 (d, $J = 6.0$ Hz, 3H, CH_3), 1.11 (d, $J = 6.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): 174.8, 168.6, 143.8, 136.9, 136.5, 135.8, 130.0, 128.9, 128.8, 128.0, 127.0, 126.5, 124.4, 121.9, 108.2, 75.5, 71.3, 46.6, 26.7, 21.7, 21.1, 21.0, 20.6; Mass (*m/z*, r.i.): 447 $(M + Na, 11)^+$, 278 (38), 225 (100); Elemental analysis: Calcd for $C_{28}H_{28}N_2O_2$: C 79.22, H 6.65, N 6.60%; Found: C 78.92, H 6.57, N 6.54%.

*4.1.2.6. 1-Diphenylmethyl-3,3-bis(4-methylphenyl)-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3f**).* (IR, KBr, cm^{-1}): 1759, 1726; ^1H NMR ($CDCl_3$, δ ppm): 7.33–6.13 (22H, arom.), 5.86 (s, 1H, methine), 2.87 (s, 3H, N–CH_3), 2.29 and 2.26 (2s, 6H, Ph-CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): 174.0, 169.0, 143.9, 138.9, 137.0, 136.5, 136.4, 136.1, 136.0, 130.0, 129.7, 128.9, 128.9, 128.1, 128.1, 127.7, 127.5, 127.3, 126.4, 124.0, 121.5, 107.8, 75.0, 71.5, 62.8, 26.3, 21.1, 21.0; Mass (*m/z*, r.i.): 571 $(M + Na, 100)^+$, 555 (25), 497 (38), 480 (42), 468 (50), 451 (52), 367 (33), 349 (32), 325 (15), 265 (25); Elemental analysis: Calcd for $C_{38}H_{32}N_2O_2$: C 83.18, H 5.88, N 5.11%; Found: C 82.74, H 5.55, N 4.91%.

*4.1.2.7. 1-(α-Phenylethyl)-3,3-bis(4-methylphenyl)-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3g**).* (IR, KBr, cm^{-1}): ^1H NMR ($CDCl_3$, δ ppm): 7.46–5.93 (17H, arom.), 4.64 (q, $J = 6.0$ Hz, 1H, methine), 2.96 (s, 3H, N–CH_3), 2.24 (s, 6H, Ph-CH_3), 1.75 (d, $J = 6.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): 174.0, 169.3, 143.9, 138.2, 136.9, 136.4, 135.8, 129.8, 128.8, 128.7, 128.3, 128.0, 127.8, 127.7, 127.2, 126.5, 123.9, 121.5, 107.8, 75.2, 71.4, 55.2, 26.3, 21.1, 21.0, 20.7; Mass (*m/z*, r.i.): 509 $(M + Na, 100)^+$; Elemental analysis: Calcd for $C_{33}H_{30}N_2O_2$: C 81.45, H 6.21, N 5.76%; Found: C 80.94, 5.78, N 5.56%.

*4.1.2.8. 1-Cyclohexyl-3,3-bis(4-methylphenyl)-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3h**).* (IR, KBr, cm^{-1}): 1750, 1720; ^1H NMR ($CDCl_3$, δ ppm): ^1H NMR ($CDCl_3$, δ ppm): 7.35–6.24 (12H, arom.), 3.36 (tt, 1H, N–CH), 3.26 (s, 3H, N–CH_3), 2.27 and 2.25 (2s, 6H, Ph-CH_3), 2.05–1.05 (m, 10H, C-hex); ^{13}C NMR ($CDCl_3$, δ ppm): 174.9, 168.6, 143.7, 136.9, 136.5, 135.8, 130.0, 128.9, 128.8, 128.0, 127.0, 126.5, 124.4, 121.9, 108.2, 75.5, 71.3, 54.3, 31.8, 30.7, 26.7, 25.2, 25.1, 21.1, 21.0; Mass (*m/z*, r.i.): 487 $(M + Na, 20)^+$, 265 (100), 247 (20), 145 (30), 118 (20); Elemental analysis: Calcd for $C_{31}H_{32}N_2O_2$: C 80.14, H 6.94, N 6.03%; Found: C 79.70, H 6.33, N 5.64%.

*4.1.2.9. 1-Isopropyl-3,3-bis(4-methoxyphenyl)-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3i**).* (IR, KBr, cm^{-1}): 1747, 1720; ^1H NMR ($CDCl_3$, δ ppm): 7.38–6.28 (12H, arom.), 3.78 (s, 6H, OCH_3), 3.71 (sept, $J = 6.0$ Hz, 1H, methine), 3.25 (s, 3H, N–CH_3), 1.25 (d, $J = 6.0$ Hz, 3H, CH_3), 1.11 (d, $J = 6.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): 174.7, 168.7, 158.6, 158.4, 143.8, 131.0, 130.9, 130.0, 129.3, 127.9, 126.9, 124.4, 122.0, 113.6, 113.5, 108.2, 74.9, 71.4, 55.2, 55.1, 46.6, 26.6, 21.7, 20.6; Mass (*m/z*, r.i.): 479 $(M + Na, 12)^+$, 225 (100); Elemental analysis: Calcd for $C_{28}H_{28}N_2O_4$: C 73.66, H 6.18, N 6.14%; Found: C 73.25, H 5.94, N 5.94%.

*4.1.2.10. 1-Diphenylmethyl-3,3-bis(4-methoxyphenyl)-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-dione (**3j**).* (IR, KBr, cm^{-1}): 1759, 1728; ^1H NMR ($CDCl_3$, δ ppm): 7.35–6.15 (22H, arom.), 5.86 (s, 1H, methine), 3.78 and 3.75 (2s, 6H, Ph-OCH_3), 2.86 (s, 3H, N–CH_3); ^{13}C NMR ($CDCl_3$, δ ppm): 174.1, 169.8, 158.7, 158.4, 143.9, 138.9, 136.4, 131.3, 130.0, 129.7, 129.4, 127.7, 127.6, 127.5, 127.3, 127.3, 124.1, 121.6, 113.6, 113.5, 107.8, 74.4, 71.6, 62.8, 55.2, 55.1, 26.3; Elemental analysis: Calcd for $C_{38}H_{32}N_2O_4$: C 78.60, H 5.55, N 4.82%; Found: C 78.80, H 5.42, N 4.77%.

4.2. Pharmacology

4.2.1. Antimicrobial activities

Using microlitre syringes, different amounts (0.5, 5, 10, 50 and 100μg) of each compound and standards were spotted on different glass-backed plates coated with silica-gel (Merck) 60 F_{254}. Nutrient agar was inoculated with respective pure microbial cultures, overlaid on the above plates and incubated appropriately. Inhibition of the bacterial and fungal growth was evaluated based on a simple and rapid bioautographic

agar overlay method using procedures described earlier [38,39].

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