Bioorganic & Medicinal Chemistry Letters 21 (2011) 5465–5469

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of spiro[indole-thiazolidine]spiro[indole-pyrans] as antimicrobial agents

Rajeev Sakhuja, Siva S. Panda, Leena Khanna, Shilpi Khurana, Subhash C. Jain *

Department of Chemistry, University of Delhi, Delhi 110 007, India

ARTICLE INFO

Article history:
Received 25 April 2011
Revised 21 June 2011
Accepted 28 June 2011
Available online 2 July 2011

Keywords:
Antibacterial
Antifungal
Antimicrobial
Spiro compounds
Cycloaddition

ABSTRACT

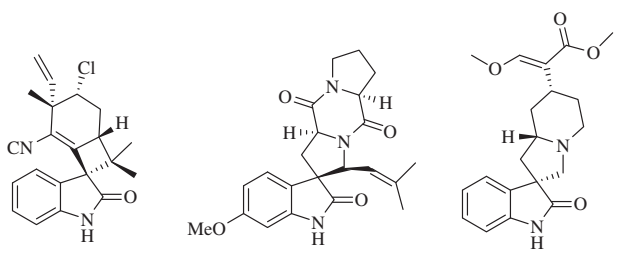
A series of novel spiro[indole-thiazolidine]spiro[indole-pyran] derivatives were synthesized from *N*-(bromoalkyl)indol-2,3-diones via monospiro-bisindole intermediates; the two indole nuclei being connected via N–$(CH_2)_n$–N linker. Synthesized compounds were evaluated for their antimicrobial activities in vitro against three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis,* and *Staphylococcus epidermis*), four Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumonia*) as well as four fungi (*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Candida albicans*) using Cup plate method. Bis spiro-indoles exhibited stronger antibacterial and antifungal efficiency than their corresponding mono spiro-indoles. Compound **10e**, the most active derivative was shown to inhibit the growth of all bacterial strains and two fungal strains (*A. niger* and *C. albicans*)

Infections caused by multi-drug resistant bacteria are of major health concern worldwide. Particularly important are infections caused by the Gram-positive bacteria *Staphylococcus aureus* and species of the genus *Enterococcus*, due to increasing incidence of infections caused by these microorganisms in hospitals and communities, and their ability of developing antibiotic resistance to multiple antibiotics. Due to some serious side effects in newly introduced antibacterial agents such as semi-synthetic streptogramins quinupristin/dalfopristin, daptomycin, the development of a diversified series of antimicrobials still remains a necessity.[1a]

Indole and its analogous are good pharmacophores for designing several chemotherapeutic reagents which exhibit wide spectrum of antimicrobial activities.[1b–e] Spiro-indoles possessing a stereogenic center at C-3 are important naturally occurring and pharmaceutically active molecules with more advanced activities.[2] Synthetic methods such as intermolecular alkylations,[3] palladium-catalyzed reactions,[4] cycloadditions,[5] and sigmatropic rearrangements[6] have been developed for the synthesis of spiro-indoles, including some naturally occurring bioactive alkaloids such as Welwitindolinone A, Spirotryprostatin A, and Rhynchophylline[7] (Fig. 1).

Spiro[indole-thiazolidines] are one of the most studied 3-spiroindole derivatives with broad spectrum of pharmacological properties such as antimicrobial,[8a] antifungal,[8b] antileukemic,[8c] and anticonvulsant.[8d] Spiro[indole-pyrans] are photochromic compounds useful in various high-tech applications.[9a] Several synthetic spiroheterocycles, containing both indole and pyran heterocycles, possess anticonvulsant, analgesic,[9b] herbicidal,[9c] and antimicrobial activities.[9d]

Bis-heterocycles separated with a suitable spacer constitute another important class of compounds with antitumor[10a] and antimicrobial[10b] activities, based on the DNA binding affinity and enzyme inhibiting actions. Biological activities are usually enhanced when different functionalities or substitutions are present on the two heterocyclic moieties in the bis-compound.[10c,d] However, the extreme steric congestion in bis-heterocycles makes the construction of all-carbon quaternary centers a formidable challenge for synthetic organic chemists.[11] Thus, we could not locate even one example in literature of a bis-heterocycle possessing two spirocyclic rings, each constructed using different heterocyclic moiety. As a part of our ongoing research on the synthesis of biologically active spiroheterocycles,[12a–e] herein we report a clean and efficient



Welwitindolinone A Spirotryprostatin A Rhynchophylline

Fig. 1. Representative examples of naturally occurring spiroindoles.

* Corresponding author. Tel.: +91 11 27667725x1387; fax: +91 11 27666605.
E-mail address: jainsc48@hotmail.com (S.C. Jain).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.06.121

methodology for the synthesis of novel spiro[indole-thiazolidine] spiro[indole-pyran] derivatives; the two spirocyclic heterocyclic systems being connected via N–$(CH_2)_n$–N linker. The synthesized compounds were evaluated for their antimicrobial activities.

N-(6-bromohexyl)indol-2,3-diones (**3a–c**) were prepared in 73–77% by the *N*-alkylation of isatins (**1a–c**) with 1,6-dibromohexane (**2**) in the presence of NaH/DMF at −10 °C following our published procedure.[12c] (Scheme 1, Table 1).[13]

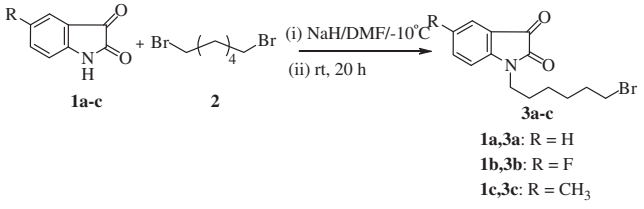
Condensation of *N*-(6-bromohexyl)indol-2,3-diones (**3a–c**) through the amino group of 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**4**) in absolute ethanol yielded corresponding Schiff base (**5a–c**) in 82–86% yield (Scheme 2, Table 2)[14] as colored solids. For **5a**, the IR spectrum showed characteristic absorption band at 1648 cm^{-1} for >C=N– stretching, thus indicated the formation of a Schiff's base. The methyl protons singlets of the pyrazoline nucleus at δ 3.26 and 2.46 in its ^1H spectrum and the imine carbon ((>C=N–) at C-3) at δ 138.1 in its ^{13}C NMR were the final evidence for the formation of azomethineylide.

Cycloaddition of mercaptoacetic acid (**6**) on **5a–c** by heating in toluene under reflux using Dean-Stark apparatus yielded spiro[indol-pyrazolin-thiazolidine]-2,4′-diones (**7a–c**) in 79–87% yield. (Scheme 2, Table 2)[15] The reaction progress was monitored by TLC. In addition, the cycloadducts were characterized by spectral analysis. For **7a**, two one proton characteristic doublets, at δ 4.49 and 4.33 (J = 14.8 Hz) for the methylene of the newly formed thiazolidinone ring supported the formation of spiro[indole-thiazolidine]-2,4′-diones.

N-alkylation of another 1*H*-indol-2,3-dione derivatives (**1a–c**) were carried using *N*-bromohexyl-spiro[indole-thiazolidine]-2,4′-diones (**7a–c**) as alkylating agents in NaH/DMF under controlled conditions (−10 °C) to obtain monospiro bis indole intermediates (**8a–i**) as colored compounds in 59–66% yields (Scheme 3, Table 3).[16] We observed that the addition of bromides **7a–c** at temperatures greater than 10 °C leads to the destruction of the thiazolidinone ring in **7a–c** and with the result the yields of the expected products were extremely low. For **8a**, a four proton multiplet at δ 3.62 for the two methylenes attached to the two nitrogen atoms of the two oxoindole system and the two one proton doublets at δ 4.38 and 4.28 (J = 14.2 Hz) were observed, indicating the formation of bisindole and conservation of spiro[indol-pyrazolinyl-thiazolidinone] system, respectively. The other spectral data further supported the formation of the products.

Finally the reaction of monospiro bis indole intermediates (**8a–i**) with double equivalents of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**9**) in absolute ethanol at room temperatures yielded spiro[indole-pyrazolinyl-thiazolidine]spiro[dipyrazolopyran-indoles] (**10a–i**) in 60–69% yield (Scheme 3, Table 3).[17] For **10a**, two three proton singlets at δ 3.01 and 2.03 and two one proton doublets at δ 4.66 and 4.22 (J = 14.6 Hz) in its ^1H NMR spectrum, accounted the existence of spiro[indol-pyrazolinyl-thiazolidine] on one of the two indole nuclei. While, two more three proton singlets at δ 2.20 and 2.10 indicated the possibility of condensing two molecules of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one as earlier mentioned in literature.[18] High resolution mass further supported the formation of two spiro heterocyclic systems on bisindolyl compound.

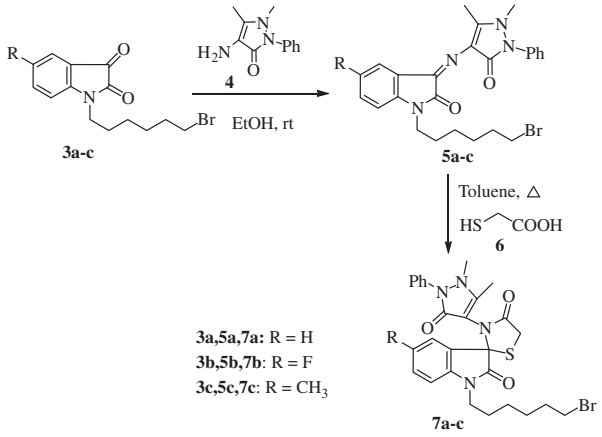
The absence of any minor signals in the ^1H NMR spectra of compounds **10a–i** and their univocal correspondence to the expected structure showed that spiro compounds containing two chiral centers are not a mixture of four possible stereoisomers but a racemate of one enantiomeric pair out of two possible pairs. Thus



Scheme 1.

Table 1
Synthesis of *N*-(6-bromohexyl)indol-2,3-diones (**3a–c**)

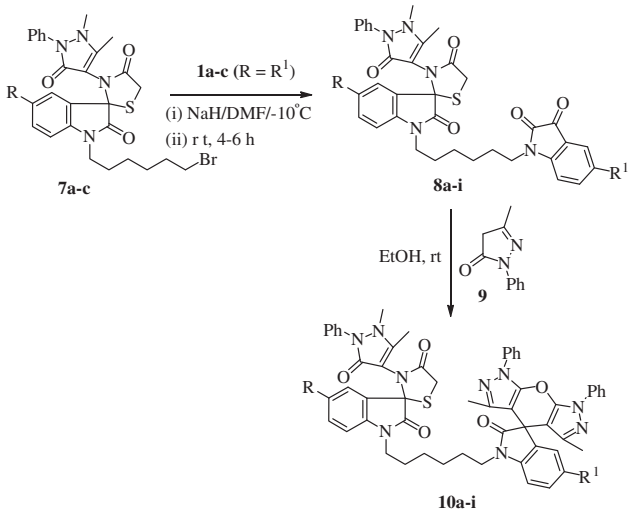
Substituent R	Product	Yield (%)	Mp (°C)	Lit. mp (°C)
H	**3a**	77	71–72	72[12c]
F	**3b**	73	76	76[12c]
CH_3	**3c**	77	64–66	–



Scheme 2.

Table 2
Synthesis of azomethine ylides (**5a–c**) and spiro [indole-thiazolidines] (**7a–c**)

Substituent **R**	Azomethine ylides			Spiro [indole-thiazolidines]		
	5	Yield (%)	mp (°C)	**7**	Yield (%)	mp (°C)
H	**5a**	85	139–140	**7a**	79	75–77
F	**5b**	82	173–175	**7b**	82	87–89
CH_3	**5c**	86	135–136	**7c**	87	93–94



Scheme 3.

Table 3
Synthesis of **8a-i** and spiro [indole-thiazolidine]-spiro[indole-pyrans] (**10a-i**)

Substituents		Monospiro bis indole			Spiro [indole-thiazolidine]-spiro[indole-pyrans]		
R	R^1	**8**	%	Mp (° C)	**10**	%	Mp (° C)
H	H	**8a**	65	98–99	**10a**	69	137–139
H	F	**8b**	62	103–105	**10b**	62	135–136
H	CH_3	**8c**	60	123–125	**10c**	68	138–139
F	H	**8d**	65	91–93	**10d**	66	133–135
F	F	**8e**	61	101–103	**10e**	62	101–103
F	CH_3	**8f**	64	105–106	**10f**	64	139–140
CH_3	H	**8g**	59	115–117	**10g**	63	135–136
CH_3	F	**8h**	66	107–108	**10h**	62	134–135
CH_3	CH_3	**8i**	63	97–98	**10i**	60	137–139

the formation of second spirocyclic ring is diastereospecific, probably because of steric hindrance. However, the products were not crystalline and thus a complete assignment of the absolute configuration at the two chiral centers is not possible at this stage.

Among all, compounds **7a–c** and **10a–i** were screened for their in vitro antimicrobial activities to determine zone of inhibition at 100 μg/mL against three Gram-positive bacteria (*S. aureus* MTCC 096, *Bacillus subtilis* MTCC 441 and *Staphylococcus epidermis* MTCC 435), four Gram-negative bacteria (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhi* MTCC 733, and *Klebsiella pneumoniae* MTCC 432) as well as four fungi (*Aspergillus niger* MTCC 282, *Aspergillus fumigatus* MTCC 343, *Aspergillus flavus* MTCC 277, and *Candida albicans* MTCC 227) using Cup plate method[19,20] where inoculated Muller-Hilton agar for bacteria and Sabouraud dextrose agar for fungi was poured onto the sterilized petri dishes (25–30 mL: each petri dish). The poured material was allowed to set (30 min.) and thereafter the 'CUPS' (08 mm diameter) was made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. Into these cups the test compound solution (0.1 mL) was added with the help of a micro pipette. The plates were incubated at 37 °C for 14 h for bacteria and 30 h for fungi and the results were noted. The test solution was prepared by DMSO as solvent. Clinically antimicrobial drugs Ciprofloxacin and Miconazole were used as the positive control and DMSO was used for blank.

The obtained results, depicted in Tables 4 and 5, revealed that spiro-indoles **7a–c** and bis spiro-indoles **10a–i** could effectively, to some extent, inhibit the growth of all tested strains in vitro. In antibacterial studies, all the compounds tested were less active towards *S. epidermis* and *P. aeruginosa* in compared to other five strains of bacteria, where as all the compounds showed moderate to comparable activity against *S. aureus* and *K. pneumonia*. Out of four strains of fungi, these spiro-indoles were showed significant activity against *A. niger* and *C. albicans*, where as moderate to mild activity against *A. fumigatus* and *A. flavus*.

Table 5
Antifungal evaluation of **7a–c** and **10a–i** (zone of inhibition in mm)

Compound	*A. niger*	*A. fumigatus*	*A. flavus*	*C. albicans*
7a	15	–	–	16
7b	16	–	14	17
7c	15	–	–	15
10a	16	13	14	17
10b	16	14	15	18
10c	17	–	15	18
10d	16	14	14	18
10e	20	15	16	21
10f	17	12	15	18
10g	16	12	12	17
10h	18	13	12	19
10i	16	14	14	18
Miconazole	18	15	17	19

The prepared mono spiro-indoles **7a–c** demonstrated moderate to good antimicrobial activities towards the tested microorganisms. Noticeably, the fluoro derivative of spiro-indoles gave comparably better activity than others against all the microorganisms. These showed that halogen-containing heterocyclic compounds exerted important influence on antimicrobial activities.

It was particularly pointed out that, among these tested bis spiro-indoles **10a–i** linked via $(CH_2)_6$ linker possessed significant antimicrobial activities with respect to standard drugs and it gave the better zone of inhibition value in compare to mono spiro-indoles. These results further indicated that moiety was specifically favorable for antimicrobial activities, which could not only broaden the antimicrobial spectrum, but also increase the bioactivity significantly. Particularly, compound **10e** could efficiently inhibit the growth of all tested Gram-positive and -negative bacteria, which was almost equipotent to Ciprofloxacin, and its antifungal potency against *A. niger* and *C. albicans* was also comparable to positive control Miconazole. It was also suggested that bis spiro-indoles

Table 4
Antibacterial evaluation of **7a-c** and **10a-i** (zone of inhibition in mm)

Compound	*S. aureus*	*B. subtilis*	*S. epidermis*	*E. coli*	*P. aeruginosa*	*S. typhi*	*K. pneumonia*
7a	14	13	–	15	14	12	13
7b	16	15	14	17	15	17	16
7c	15	14	12	15	–	15	15
10a	16	15	13	16	–	16	15
10b	17	16	15	16	16	16	17
10c	15	15	–	16	12	15	16
10d	16	17	15	17	16	18	17
10e	19	19	16	19	19	20	19
10f	15	16	15	17	16	16	17
10g	15	15	13	15	15	17	18
10h	18	16	12	15	17	19	18
10i	17	14	14	14	15	17	16
Ciprofloxacin	19	18	16	20	18	19	17

10a–i were worthy for further investigations as potential antimicrobial agent.

For tested mono spiro-indoles **7a–c** and bis spiro-indoles **10a–i**, the structural parameters of spacers including substituents, the types of linkers and the lengths of aliphatic chains markedly influenced their antimicrobial efficacy. Moreover, the antimicrobial potency for spiro-indoles **7a–c** and **10a–i**, depended on the substituent and lengths of aliphatic chains, and the activities seemed to decrease with the increase of aliphatic chain length. Obviously, the bridged linkers have large effect on their antimicrobial efficiency, and further works are essential in order to deduce the structure–activity relationship.

Twenty five novel heterocycles including the intermediates were synthesized in good to excellent yields and fully characterized on the basis of their detailed spectral studies. In summary, we have developed a clean and efficient methodology for the synthesis of novel spiro[indole-thiazolidine]spiro[indole-pyran] derivatives; the two spirocyclic heterocyclic systems being connected via N–$(CH_2)_n$–N linker using molecular modification approach. The methodology could be further extended and used for the synthesis of other asymmetric bis(spiro-heterocycles) of biological importance.

Acknowledgments

Authors (R.S. and S.K.) and author (S.S.P.) are thankful, respectively, to CSIR and UGC, New Delhi for research fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.121.

References and notes

1. (a) Leclercq, R. *Clin. Microbiol. Infect.* **2009**, *15*, 224; (b) Zhang, W.; Locurcio, M.; Lin, C. C.; Jimenez, L. S. *J. Heter. Chem.* **1996**, *33*, 1647; (c) Le Borgne, M.; Marchand, P.; Duflos, M.; Delevoyl-Seiller, B.; Piessard-Robert, S.; Le Baut, G.; Hartmann, R. W.; Palzer, M. *Arch. Pharm. Med. Chem.* **1997**, *330*, 141; (d) Scott, R. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723; (e) Varvarescu, A.; Tsantili-Kakouliduo, A.; Siatrapapastaikoudi, T.; Tiligada, E. *Arzneim.-Forsch./Drug Res.* **2000**, *50*, 48.
2. (a) Marti, C.; Carreira, E. M. *Eur. J. Org. Chem.* **2003**, 2209; (b) Tsuda, M.; Mugishima, T.; Komatzu, K.; Sone, T.; Tanaka, M.; Mikaimi, Y.; Shiro, M.; Hirai, M.; Ohizumii, Y.; Kobayashi, J. *Tetrahedron* **2003**, *59*, 3227; (c) Thericke, R.; Tang, Y. Q.; Sattler, I.; Grabley, S.; Feng, X. Z. *Eur. J. Org. Chem.* **2001**, 261; (d) Jossang, A.; Jossang, P.; Hadi, H. A.; Sevenet, T.; Bodo, B. *J. Org. Chem.* **1991**, *56*, 6527; (e) Zaveri, N. T.; Jiang, F.; Olsen, C. M.; Deschamps, J. R.; Parrish, D.; Polgar, W.; Toll, L. *J. Med. Chem.* **2004**, *47*, 2973.
3. (a) Huang, A.; Kodanko, J. J.; Overman, L. E. *J. Am. Chem. Soc.* **2004**, *126*, 14043; (b) Bagul, T. D.; Lakshmaiah, G.; Kawabata, T.; Fuji, K. *Org. Lett.* **2002**, *4*, 249.
4. Ruck, R. T.; Huffman, M. A.; Kim, M. M.; Shevlin, M.; Kandur, W. V.; Davies, I. W. *Angew. Chem. Int. Ed.* **2008**, *47*, 4711.
5. (a) Yong, S. R.; Williams, M. C.; Pyne, S. G.; Ung, A. T.; Skelton, B. W.; White, A. H.; Turner, P. *Tetrahedron* **2005**, *61*, 8120; (b) Beccalli, E. M.; Clerici, F.; Gelmi, M. L. *Tetrahedron* **2003**, *59*, 4615.
6. (a) Kawasaki, T.; Ogawa, A.; Terashima, R.; Saheki, T.; Ban, N.; Sekiguchi, H.; Sakaguchi, K.; Sakamoto, M. *J. Org. Chem.* **2005**, *70*, 2957; (b) Mao, Z.; Baldwin, S. W. *Org. Lett.* **2004**, *6*, 2425.
7. (a) Stratmann, K.; Moore, R. E.; Bonjouklian, R.; Deeter, J. B.; Patterson, G. M. L.; Shaffer, S.; Smith, C. D.; Smitka, T. A. *J. Am. Chem. Soc.* **1994**, *116*, 9935; (b) Edmondson, S.; Danishefsky, S. J.; Sepp-Lorenzino, L.; Rosen, N. *J. Am. Chem. Soc.* **1999**, *121*, 2147.
8. (a) Mhaske, P. C.; Shelke, S. H.; Jadhav, R. P.; Raundal, H. N.; Patil, S. V.; Patil, A. A.; Bobade, V. D. *J. Heterocycl. Chem.* **2010**, *47*, 1415; (b) Dandia, A.; Singh, R.; Khaturia, S.; Merienne, C.; Morgant, G.; Loupy, A. *Bioorg. Med. Chem.* **2006**, *14*, 2409; (c) Rajopadhye, M.; Popp, F. D. *J. Heterocycl. Chem.* **1987**, *24*, 1637; (d) Rajopadhye, M.; Popp, F. D. *J. Heterocycl. Chem.* **1984**, *21*, 289.
9. (a) Abe Abe, Y.; Ebara, H.; Okada, S.; Akaki, R.; Horii, T.; Nakao, R. *Dyes and Pigments* **2002**, *52*, 23; (b) Joshi, K. C.; Jain, R.; Sharma, K.; Bhattacharya, S. K.; Goel, R. K. *J. Indian Chem. Soc.* **1988**, *65*, 202; (c) Joshi, K. C.; Jain, R.; Arora, S. *J. Indian Chem. Soc.* **1988**, *65*, 277; (d) Nandakumar, A.; Thirumurugan, P.; Perumal, P. T.; Vembu, P.; Ponnuswamy, M. N.; Ramesh, P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4252.
10. (a) Thurston, D. E.; Bose, D. S.; Thompson, A. S.; Howard, P. W.; Leoni, A.; Croker, S. J.; Jenkins, T. C.; Neidle, S.; Hartley, J. A.; Hurley, L. H. *J. Org. Chem.* **1996**, *61*, 8141; (b) Shaker, R. M. *Phosphorus, Sulfur Silicon Relat. Elem.* **1999**, *149*, 7; (c) Kamal, A.; Laxman, N.; Ramesh, G.; Neelima, K.; Kondapi, A. K. *Chem. Commun.* **2001**, 437; (d) Raasch, A.; Scharfenstein, O.; Traenkle, C.; Holzgrabe, U.; Mohr, K. *J. Med. Chem.* **2002**, *45*, 3809.
11. Christoffers, J.; Mann, A. *Angew. Chem. Int. Ed.* **2001**, *40*, 4591.
12. (a) Jain, S. C.; Bhagat, S.; Rajwanshi, V. K.; Babu, B. R.; Sinha, J. *Indian J. Chem. B* **1997**, *36B*, 633; (b) Jain, M.; Khanna, P.; Saxena, A.; Bhagat, S.; Olsen, C. E.; Jain, S. C. *Synth. Commun.* **2006**, *36*, 1863; (c) Jain, M.; Sakhuja, R.; Khanna, P.; Bhagat, S.; Jain, S. C. *Arkivoc* **2008**, 54; (d) Jain, S. C.; Khanna, P.; Bhagat, S.; Jain, M.; Sakhuja, R. *Phosphorus, Sulphur Silicon Relat. Elem.* **2005**, *180*, 1829; (e) Khanna, P.; Saxena, A.; Khanna, L.; Bhagat, S.; Jain, S. C. *Arkivoc* **2009**, 119.
13. Representative procedure for 1-(6-bromohexyl)-5-methylindoline-2,3-dione (**3c**): To a stirred solution of sodium hydride (40 mmol) in DMF (10 mL) was added dropwise a solution of 5-methyl-1*H*-indol-2,3-dione (**1c**; 30 mmol) in DMF (10 mL) during 20 min at −10 °C, under inert atmosphere of nitrogen. To the deep purple colored suspension that resulted, 1,6-dibromohexane (**2**; 30 mmol) was added dropwise at the same temperature. The contents were allowed to stir overnight at room temperature and thereafter quenched with ice water to yield a red colored semisolid. This was extracted with dichloromethane (3 × 50 mL) and the organic layer was purified by flash column chromatography in petroleum ether/dichloromethane (70:30) as eluant to yield pure **3c** as red microcrystals; IR $_{max}$ (KBr): 2936, 2854, 1737, 1620, 1610, 1482, 1463, 1347, 1260, and 1220 cm^{-1}.^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.32 (m, 2H), 6.81 (m, 1H), 3.76 (t, *J* = 6.8 Hz, 2H), 3.38 (t, *J* = 6.9 Hz, 2H), 2.20 (s, 3H), 1.83 (m, 2H), 1.71 (m, 2H), 1.46 (m, 4H).^{13}C NMR (75.47 MHz, $CDCl_3$): δ_C 183.8, 174.0, 154.8, 137.1, 128.1, 126.0, 115.1, 113.0, 41.6, 34.6, 33.2, 29.7, 27.8, 27.6, 21.4. EIMS *m/z* (%): 325 (M^++2, 28), 323 (M^+, 30), 297 (15), 244 (M^+-Br, 12), 217 (50), 190 (38). Anal. Calcd for $C_{15}H_{18}BrNO_2$: C, 55.57; H, 5.60; N, 4.32. Found: C, 55.72; H, 5.48; N, 4.43. This procedure for followed for all the substrates.
14. Representative procedure for 1-(6-bromohexyl)-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)imino)indolin-2-one (**5a**): A mixture *N*-(6-bromohexyl)indol-2,3-dione (**3a**; 9.5 mmol) and 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**4**; 9.5 mmol) in absolute ethanol (20 mL) was stirred at room temperature for 2 h. The colored solids thus obtained were filtered and recrystallized from dichloromethane to yield Schiff's base (**5a**) as orange solid; IR $_{max}$ (KBr): 2931, 2858, 1714, 1648, 1605, 1548, 1487, 1432, 1357, 1310, and 1247 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.44 (m, 5H), 7.34 (m, 2H), 7.05 (t, *J* = 7.3 Hz, 1H), 6.81 (d, *J* = 8.2 Hz,1H), 3.77 (t, *J* = 6.9 Hz, 2H), 3.39 (t, *J* = 6.8 Hz, 2H), 3.26 (s, 3H), 2.46 (s, 3H), 1.86 (m, 2H), 1.82 (m, 2H), 1.46 (m, 4H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ_C 171.2 (C-2), 161.3, 138.1 (C-3), 132.1–108.2 (aromatic carbons), 39.9, 35.8, 33.6, 32.5, 27.7, 27.5, 27.2, 11.3. EIMS *m/z* (%): 496 (M^++2, 60), 494 (M^+, 58), 450 (12), 415 (M^+-Br, 6), 291 (32), 240 (19). Anal. Calcd for $C_{25}H_{27}BrN_4O_2$: C, 60.61; H, 5.49; N, 11.31. Found: C, 60.48; H, 5.56; N, 11.29. This procedure for followed for all the substrates.
15. Representative procedure for 1-(6-bromohexyl)-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)spiro[indoline-3,2-thiazolidine]-2,4-dione (**7a**): A mixture of 1-(6-bromohexyl)-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)imino)indolin-2-one (**5a**; 6 mmol) and mercaptoacetic acid (**6**) (6.5 mmol) was heated under reflux in dry toluene for 9 h, with azeotropic removal of water simultaneously, using Dean Stark apparatus. The reaction mixture turned light yellow in color and a sticky yellow solid was formed. The toluene was distilled out and the oily mixture left was treated with saturated solution of sodium bicarbonate to remove excess acid. The solid obtained was stirred for an hour at room temperature and filtered, dried and recrystallized from dichloromethane/methanol to obtain spiro[indol-pyrazolin-thiazolidine]-2,4-dione (**7a**) as dirty white solid; IR $_{max}$ (KBr): 2932, 2857, 1720, 1701, 1684, 1611, 1489, 1467, 1359, 1285, and 1209 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.36 (m, 2H), 7.27 (m, 3H), 7.18 (m, 3H), 6.74 (d, *J* = 7.8 Hz, 1H), 4.49 & 4.33 (2d, *J* = 14.8 Hz, 1H each), 3.69 (t, *J* = 6.8 Hz, 2H), 3.39 (t, *J* = 6.9 Hz, 2H), 3.01 (s, 3H), 2.14 (s, 3H), 1.84 (m, 2H), 1.68 (m, 2H), 1.48 (m, 2H), 1.39 (m, 2H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ_C 177.4, 172.1, 162.2, 134.4–108.0 (aromatic carbons), 67.3, 40.1, 35.0, 33.4, 32.4, 27.6, 26.9, 26.3, 25.8, 10.7. EIMS *m/z* (%): 570 (M^++2, 24), 568 (M^+, 23), 506 (36), 489 (100), 433 (56), 341 (17), 260 (30), 245 (32), 203 (31), 190 (48). Anal. Calcd for $C_{27}H_{29}BrN_4O_3S$: C, 56.94; H, 5.13; N, 9.84. Found: C, 56.76; H, 5.19; N, 10.04. This procedure for followed for all the substrates.
16. Representative procedure for 3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazo-4-yl)-1-(6-(2,3-dioxoindolin-1-yl)hexyl)spiro[indoline-3,2-thiazolidine]-2,4-dione (**8a**): To a stirred solution of sodium hydride (1.08 mmol) in DMF (10 mL) was added dropwise solution of 1*H*-indol-2,3-dione (**1a**; 0.9 mmol) in DMF (10 mL) during 20 min at −10 °C under inert atmosphere of nitrogen. To the deep purple colored suspension that resulted, spiro[indol-pyrazolin-thiazolidine]-2,4-dione (**7a**; 0.9 mmol) was added dropwise at the same temperature. The contents were allowed to stir at room temperature for 4–6 h and thereafter quenched with ice water to yield colored solids, which were filtered and recrystallized from dichloromethane/methanol to give pure monospiro bisindoles (**8a**) intermediate as deep orange needles; IR $_{max}$ (KBr): 2929, 2857, 1725, 1701, 1684, 1611, 1488, 1467, 1355, and 1164 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.97 (m, 1H), 7.59 (m, 2H), 7.32 (m, 3H), 7.17–7.10 (m, 5H), 6.85 (m, 1H), 6.74 (d, *J* = 6.7 Hz, 1H), 4.38 and 4.28 (2d, *J* = 14.2 Hz, 1H each), 3.62 (m, 4H), 2.95 (s, 3H), 2.12 (s, 3H), 1.68 (m, 4H), 1.42 (m, 4H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ_C 180.1, 175.2, 172.3, 158.0, 154.2–108.0 (aromatic carbons), 69.2, 39.8, 34.9, 32.8, 32.2, 29.5, 26.9, 26.3, 10.7. Anal. Calcd for $C_{35}H_{33}N_5O_5S$: C, 66.12; H, 5.23; N, 11.02. Found: C, 66.38; H, 5.62; N, 10.73. This procedure for followed for all the substrates.

17. Representative procedure for 1-(6-(3,5-dimethyl-2-oxo-1,7-diphenyl-1,7-dihydrospiro[indoline-3,4-pyrano[2,3-*c*:6,5-*c*]dipyrazol]-1-yl)hexyl)-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)spiro[indoline-3,2-thiazolidine]-2,4-dione (**10a**): A solution of monospiro bisindoles (**8a–i**; 1.5 mmol) and 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**9**; 3.01 mmol) was stirred in absolute ethanol for about 8–10 h at room temperature, till the color of reaction mixture fades and a dirty white solid separated out. The solid thus precipitated was filtered, dried and purified by flash column chromatography using dichloromethane/methanol (97:3) to yield spiro[indole-thiazolidine] spiro[indole-pyrans] (**10a–i**) as white solid; IR $_{max}$ (KBr): 2928, 2854, 1717, 1611, 1493, 1361, 1306, 1284, and 1160 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 7.98 (m, 1H), 7.96 (m, 1H), 7.87 (m, 1H), 7.53 (m, 5H), 7.38 (m, 4H), 7.29 (m, 3H), 7.19 (m, 5H), 7.03 (m, 1H), 6.81 (m, 1H), 6.78 (m, 1H), 4.66 and 4.22 (2d, J = 14.6 Hz, 1H each), 3.77 (m, 4H), 3.01 (s, 3H), 2.20 & 2.10 (2s, 3H each), 2.03 (s, 3H), 1.86 (m, 2H), 1.72 (m, 2H), 1.60 (m, 4H). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ_C 175.1, 172.2, 158.6, 153.2–108.6 (aromatic carbons), 67.3, 50.9, 40.5, 35.2, 33.2, 27.3, 26.6, 17.9, 17.1, 11.2. HRMS: m/z $[M + H]^+$ calcd for $C_{55}H_{50}N_9O_5S$: 948.3650; found: 948.3662. This procedure for followed for all the substrates.
18. Joshi, K. C.; Pardasani, R. T.; Dandia, A.; Bhagat, S. *Heterocycles* **1991**, *32*, 1491.
19. Barry, A. L. In *The Antimicrobic Susceptibility Test: Principles and Practices*; Lea & Febiger: Philadelphia, 1976; p 180.
20. Reddy, P. M.; Ho, Y. P.; Shanker, K.; Rohini, R.; Ravinder, V. *Eur. J. Med. Chem.* **2009**, *44*, 2621.