Polyethylene Glycol Mediated, One-Pot, Three-Component Synthetic Protocol for Novel 3-[3-Substituted-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-ones as New Class of Potential Antimicrobial and Antitubercular Agents

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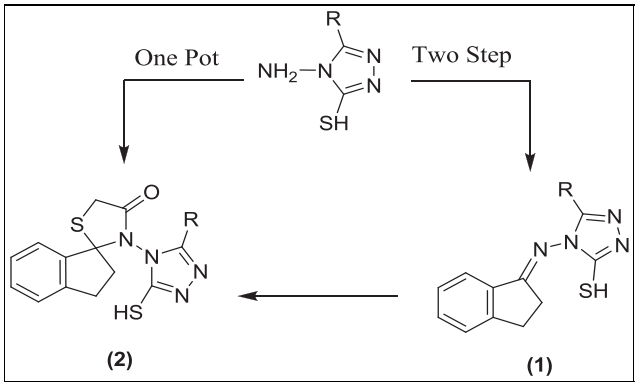

A series of 3-[3-substituted-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-ones **2** were designed for the purpose of searching for novel antimicrobial agents and have been synthesized conveniently in a single step with a three-component protocol in polyethylene (400) as green reaction media. Thus, the condensation reaction between indane-1-one; 4-amino-5-mercapto-1,2,4-triazoles and mercaptoacetic acid in polyethylene glycol (400) gave quantitatively and analytically pure titled compounds **2**. The structure of synthesized compound **2** is based on spectral (IR, ^{1}H-NMR, and ^{13}C-NMR) as well elemental analyses. These compounds have been screened for their antibacterial, antifungal, and antitubercular activities. Some of them have showed significant inhibition on fungal and bacterial growth and antitubercular activity against *Mycobacterium tuberculosis*. The compounds **2a**, **2d**, and **2e** display antifungal activity against *Candida albicans* [minimum inhibitory concentration (MIC) 3.13, 6.25 μg/mL] and antibacterial activity against *Streptococcus pneumoniae* (MIC 3.13 μg/mL) of the order of standard drugs tested under similar conditions, and compound **2a** showed better antitubercular activity than other compounds against *M. tuberculosis* (H_{37}Rv strain, MIC 12.5 μg/mL).

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INTRODUCTION

Antibiotics are among the most prescribed drugs in the world today. Several bacterial infections such as diarrhea, food poisoning, and rheumatic, salmonellosis extraintestinal, and intestinal wall infections are caused by multidrug resistant Gram-positive and Gram-negative pathogens [1,2]. This resistance of pathogenic bacteria toward available antibiotics is rapidly becoming a major threat worldwide to human health. In addition, the primary and opportunistic fungal infections continue to increase dramatically because of the growing number of immunocompromised hosts such as AIDS patients or those undergoing anticancer chemotherapy or transplantations [3–5]. Not only this, they also easily gained resistance, which is the main problem encountered in developing safe and efficient antifungal agents. Therefore, design of new antimicrobial compounds in structural classes to deal with these problems is of prime interest.

Thiazolidinone, an important class of sulfur-containing and nitrogen-containing heterocycles, has received extensive interest in the recent past owing to their clinical use and wide application as toxic agents in cells of pathogenic organism [6–12]. Among them, several thiazolidinone derivatives were designed and synthesized as antibacterial [13], antifungal [14], anticonvulsant [15], anti-HIV [16], and antitubercular [17] agents. Similarly, the importance of 1,2,4-triazoles as versatile pharmacodynamic moiety has been well documented in many of its derivatives, which exhibited anti-inflammatory [18], antiviral [19], antimycotic [20], and antimicrobial activities [21]. In addition, a series of 1,2,4-triazole derivatives have been patented and extensively employed in agriculture [22].

The union of heterocyclic rings through a spiro carbon atom often results compounds with interesting biological activities [23–26]. In light of these observations and continuing our ongoing research on novel heterocycles [27–31] with

biological interest, we consider it of interest to combine aforesaid heterocyclic nuclei with indane ring [32] through a spiro carbon atom in a single molecular framework **2** to see their additive effect toward biological activities of titled compounds. Several groups have been substituted at position-3 in the triazole ring to investigate the influence of such structural variation on anticipated biological activities. This investigation further appeared interesting because the titled compounds **2** may serve as a very suitable ligand to chelate essential metals present in fungi cells [33] as proposed in Figure 1. Formation of such metal chelates will increase the hydrophobic properties of metal ions, and this enables them to pass through lipoid layers of cellular membrane to the fungus cell, thereby leading to their poisoning [34].

Recently [35,36], PEG and its monomethyl ethers have emerged as an alternative green reaction media with unique properties such as thermal stability, commercial availability, nonvolatility, immiscibility with a number of organic solvents, and recyclability. On the other hand, PEGs are inexpensive, nonhalogenated, and easily degradable and possess low toxicity [37]. These properties of PEGs are making them as green reaction media in organic synthesis.

Therefore, the development of facile and convenient synthetic route to achieve rapid access to these novel spiroheterocycles is of prime interest. Thus, in the present investigation, the titled compounds **2** were synthesized by one-pot, three-component green protocol and evaluated for their antimicrobial and antitubercular activity.

RESULTS AND DISCUSSION

Synthesis. The starting material 4-amino-3-substituted-5-mercapto-[1,2,4]-triazoles was prepared from corresponding acid hydrazides, CS_2, and KOH as reported in literature to obtain potassium salt, which was reacted with hydrazine hydrate, followed by acidification with dil. HCl that afforded the triazoles. The one-pot reaction between 4-amino-5-mercapto-1,2,4-triazoles, indan-1-one, and mercaptoacetic acid in polyethylene glycol at 40°C yielded **2a–h** (Scheme 1). The reaction was supposed to occur via the formation of intermediate Schiff bases (**1a–h**) by the condensation reaction between indan-1-one and 4-amino-3-substituted-5-mercapto-[1,2,4]-triazoles followed by [3 + 2] annulation of mercaptoacetic acid with Schiff base to afford the corresponding compounds **2a–h** in quantitative yields. The authenticity of this speculation was confirmed by an experiment in which the intermediates **1a–h** were isolated, characterized, and then subjected to react with mercaptoacetic acid under [3 + 2] annulation reaction in polyethylene glycol to give **2a–h**. Superimposable IR, ^{1}H-NMR, ^{13}C-NMR spectra as well as mixed melting points and elemental analysis confirmed the authenticity of the final products formed by using one-step and two-step methods. However, the reported yield of the final products is by the use of the one-step process.

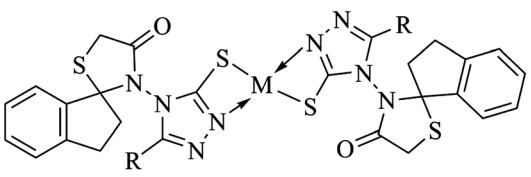


Figure 1. Chelation property.

Spectral studies. The structures of Schiff bases (**1a**−**h**) were identified from IR spectra, which showed absorption bands at 1602–1576 cm^{-1} attributed to imine (C═N), bands at 1520–1563 cm^{-1} attributed to C═N of the 1,2,4-triazole nucleus, and a characteristic band at 2750–2778 cm^{-1} attributed to -SH. The structure was further supported by ^{1}H-NMR spectrum exhibiting signals at δ 2.78–3.09 (-SH), 6.81–7.93 (aromatic H), and 1.79–2.39 (methylene H). The ^{13}C-NMR spectrum of Schiff bases (**1a–h**) showed signals at δ 152.3–157.4 due to imine carbon. The signals that appeared at δ 20.3–32.4 are assigned to methylene carbon. Signals at δ 64.5–67.2 are due to -OCH_2-. The aromatic carbon appeared in the range of δ 120.0–149.8.

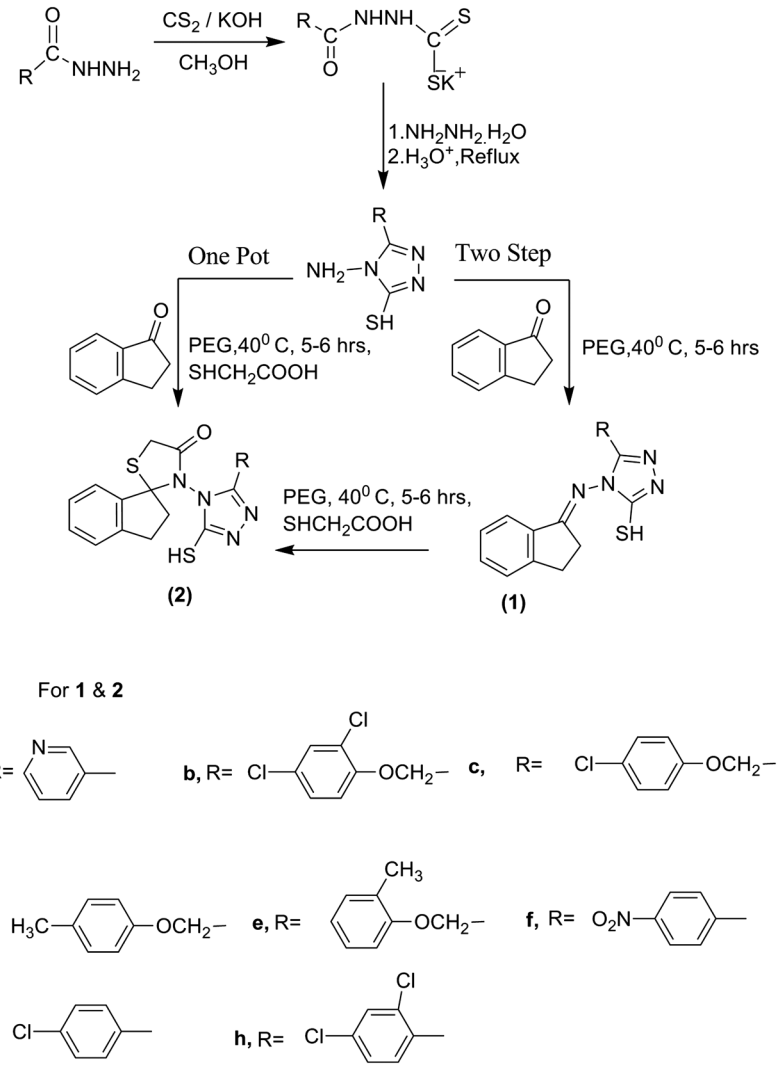
The IR spectrum of **2a–h** exhibited the absorption of new bands at 1701–1714 cm^{-1} attributed to the C═O group and the appearance of peaks at 703–689 cm^{-1} attributed to C-S-C, which clearly indicates the formation of titled compounds. ^{1}H-NMR spectrum exhibiting new signals at δ 3.63–3.91 (CH_2, thiazolidinone ring) clearly supports that the annulation took place between Schiff base and mercaptoacetic acid to obtain the titled compounds (**2a–h**). Further, ^{13}C-NMR spectrum of **2a–h** is in good agreement with structures of titled compounds. In ^{13}C-NMR spectrum, three new signals at δ 184.3–190.2 attributed to C═O carbon, at δ 68.1–71.2 attributed to spirocarbon, and at δ 34.6–38.5 for CH_2 of thiazolidinone ring appeared. The aromatic carbon appeared in the range of δ 121.2–149.4 ppm.

Antimicrobial activity

Antibacterial activity. The newly synthesized compounds (**2a–h**) were screened for their antibacterial activity against Gram-positive bacteria, *S. pneumoniae* and *Bacillus subtilis*, and Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, by disk diffusion method [38].

The antibacterial data (Table 1, Fig. 2.) revealed that all tested compound of this investigation are moderately to highly active against all the pathogenic bacteria. The compounds **2a**, **2d**, and **2e** were highly active against all pathogenic bacteria, whereas the rest of the compounds showed moderate activity as compared with the standard drug Ciprofloxacin. The data also revealed that the activity of compound **2a** > **2e** > **2d**. The different substituents on the aromatic ring exert a significance influence on the antibacterial activity. The presence of electron donating methyl group increases activity in general while its orientation at position-2, for example, **2e** worked better than at position-4, for example, **2d**. The presence of electron

Scheme 1. Synthetic route for intermediate and final compounds.



withdrawing groups (halogen and nitro) on the aromatic ring decreases the antibacterial activity as compared with the standard drug. The most active compound was **2a**, which contains a pyridine ring in place of phenyl moiety. This moiety is additive toward antibacterial activity in this class of compounds. It is to be noted that compounds **2a**, **2d**, and **2e** are more potent against Gram-positive than Gram-negative bacteria.

Antifungal activity. Newly prepared compounds were screened for their antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, and *Aspergillus flavus* (recultured) in DMSO by serial plate dilution method [39].

The antifungal screening data (Table 1, Fig. 3) revealed that all tested compounds showed moderate to strong antifungal activity, but compounds **2a**, **2c**, **2d**, **2e**, and **2h** emerged as very active agents against all the tested fungal strains. The data also revealed that the activity of compounds **2a** > **2d** > **2e** > **2h** > **2c**. On the other hand, the nature of the substituents is critical to antifungal activity. The compounds **2c** and **2h** contain electron withdrawing groups 2-chlorophenoxy and 2,4-dichlorophenyl moiety, respectively, in their structures, whereas **2d** and **2e** contains electron donating methyl group at position-4 and position-2 on the phenyl ring. This shows that the presence of electron donating group increases the antifungal activity as compared with electron withdrawing groups (halogen and nitro) in this class of compounds. It is notable that the entire tested compounds are more active on *C. albicans* than other fungal strains. We were expecting that the chelation behavior of the synthesized compound will increase the antifungal activity, but it is not found so.

Among these, the most active compound **2a** contains a pyridine ring and showed activity, which is comparable with the standard compound tested under similar condition. This

Table 1

Antimicrobial and antitubercular activity of synthesized compounds.

Compound	Antibacterial activity				Antifungal activity			Antitubercular activity
	Streptococcus pneumoniae	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus fumigatus*	*Aspergillus flavus*	*Mycobacterium tuberculosis* (H_{37}Rv strain)
2a	38 (3.13)	31 (12.5)	30 (6.25)	27 (6.25)	32 (3.13)	29 (12.5)	29 (6.25)	12.5
2b	20 (12.5)	19 (25)	21 (12.5)	23 (6.25)	21 (12.5)	20 (25)	19 (12.5)	>12.5
2c	21 (12.5)	20 (6.25)	19 (12.5)	21 (25)	22 (12.5)	20 (25)	21 (12.5)	>12.5
2d	27 (3.13)	26 (6.25)	25 (12.5)	22 (12.5)	27 (3.13)	25 (6.25)	26 (12.5)	>12.5
2e	38 (3.13)	31 (6.25)	27 (12.5)	26 (25.0)	29 (6.25)	28 (12.5)	27 (25)	>12.5
2f	17 (50)	20 (12.5)	19 (12.5)	14 (50)	20 (25)	19 (25)	22 (12.5)	>12.5
2g	23 (6.25)	21 (25)	18 (25)	20 (50)	19 (25)	21 (12.5)	20 (12.5)	>12.5
2h	22 (6.25)	20 (25)	20 (12.5)	21 (12.5)	26 (6.25)	24 (6.25)	25 (12.5)	>12.5
Ciprofloxacin	38 (3.13)	37 (6.25)	37 (3.13)	38 (3.13)	–	–	–	–
Fluconazole	–	–	–	–	32 (3.13)	28 (6.25)	30 (6.25)	–
Isoniazid (INH)	–	–	–	–	–	–	–	0.75

Zones of inhibition expressed in mm and minimum inhibitory concentrations (μg/mL) were given in brackets.

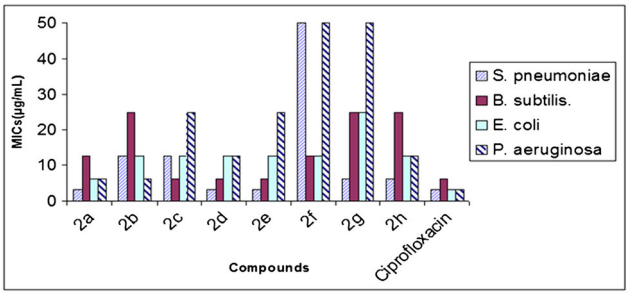


Figure 2. Antibacterial activities of synthesized compounds and standard drug. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

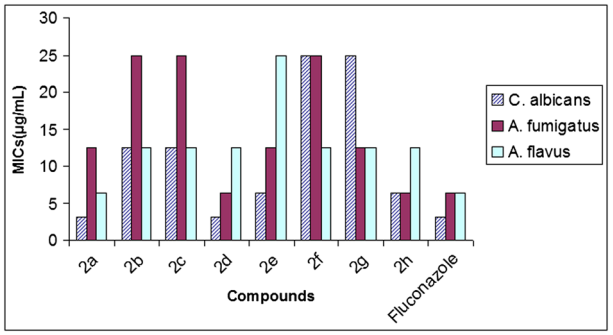


Figure 3. Antifungal activities of synthesized compounds and standard drug. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

indicates that the incorporation of two or more heterocyclic moieties in spiro association of indane with thiazolidinone increases the fungicidal activity of this class of compounds. Further investigation of these compounds on wider range of fungi as well as on more dilution is in process.

Antitubercular activity. The synthesized compounds were screened for their antitubercular activity against *Mycobacterium tuberculosis* (H_{37}Rv) by Agar microdilution technique [40]. The results of antitubercular activity were listed in Table 1. Biological results of the synthesized compounds reveal that compound **2a** showed a minimum inhibitory concentration (MIC) value 12.5 μg/mL, which is better than the other compounds. Compound **2a** containing pyridine ring in place of phenyl ring at position-3 of triazole nucleus is highly active. This suggests that the presence of pyridyl moiety enhances the activity against *M. tuberculosis* among all the compounds tested.

In conclusion, this article reports a simple, convenient, one-pot, three-component green synthesis of novel spiro (indan-1′,2-thiazolidin)-4-ones in good yields. This work has also demonstrated that spiro association of indane with 4-thiazolidinone containing other nitrogen heterocycles (1,2,4-triazole and pyridine) showed promising activity against selected fungi, bacteria, and *M. tuberculosis*. However, the nature of the substituent on position-3 of triazole nucleus, viz. pyridine, 2-methylphenoxy, 4-methylphenoxy, 2,4-dichlorophenoxy, 4-chlorophenoxy, 4-chlorophenyl, and 2,4-dichlorophenyl, is determinant for the nature and extent of the activity of the synthesized compounds, which might influence on their mode of actions. Further development of this group of compounds may lead to compounds with better antimicrobial and antitubercular profile than standard drugs by substituting a series of electron donating and heterocyclic moieties and selectively modifying the spiro system.

EXPERIMENTAL

General Procedure for one typical case for each step has been described. Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded in KBr on a Shimadzu (Japan) 8201 PC spectrophotometer (ν_{max} in cm^{-1}) and ^{1}H-NMR and ^{13}C-NMR spectra in DMSO-d_6 on a Bruker (Switzerland) DRX-300 (300 MHz) spectrometer using TMS as a internal reference (chemical shifts in δ, ppm). The purity of compounds was checked by thin layer chromatography on silica gel plate using ether, and spots were visualized with iodine chamber and UV.

General method for the synthesis of 4-amino-3-pyridyl-5-mercapto-[1,2,4]triazole. This was prepared by the method of Tomayo *et al*. [41]. Carbon disulfide (2.7 mL, 38.4 mmol) was slowly added in methanolic solution of appropriate pyridylacetohydrazine (5 g, 36.5 mmol) and KOH (2.86 g, 36.5 mmol) with constant stirring. This solution was stirred further for 2–3 h and left overnight. The mixture was then treated with hydrazine hydrate (2.2 mL, 36.5 mmol) and refluxed for 4 h. The resulting mixture was cooled and filtered. The filtrate was acidified with dil. HCl to obtain the triazoles, which were recrystallized from aq. ethanol. Similarly, the other triazoles were prepared. The prepared compounds are known and have used for further reaction.

One-pot general method for the synthesis of 2-(3-pyridyl-5-mercapto-1,2,4-triazol-4-yl)-spiro-(indan-1,2-thiazolidin)4-ones 2a. A mixture of 4-amino-3-pyridyl-5-mercapto-1,2,4-triazole (1.93 g, 10 mmol), indan-1-one (1.32 g, 10 mmol), and mercaptoacetic acid (0.77 mL, 7.6 mmol) in polyethylene glycol-400 (15 mL) was heated at 40°C for 5–6 h. The reaction mixture was poured into ice water and washed with sodium bicarbonate solution to remove excess of mercaptoacetic acid. The product thus obtained was filtered, dried, and recrystallized from dioxan : water (2.40 g, 63%). Similarly, the other compounds were prepared.

Two-step method

General method for the synthesis of 4-(indan-1-ylideneamino)-3-pyridyl-5-mercapto-[1,2,4]triazoles 1a-1h. 4-Amino-3-pyridyl-5-mercapto-[1,2,4]-triazole (1.93 g, 10 mmol) and indan-1-one (1.32 g, 10 mmol) were heated at 40°C in polyethylene glycol (15 mL) for 5–6 h. The solvent was removed and the reaction mixture was poured into ice cold water, the crude product thus obtained was filtered, dried, and recrystallized from dioxan : water (1.98 g, 65%). Similarly, the other compounds were prepared.

4-(Indan-1-ylideneamino)-3-pyridyl-5-mercapto-[1,2,4]triazole ***(1a).*** Yield, 65%; mp, 226–229 (°C); IR (KBr, cm^{-1}): 2750 (-SH), 1585 (>C═N), 1558, 1520, 1484 (C═C), 1083 (C-N-C); ^{1}H-NMR (DMSO-d_6) δ: 8.01–8.82 (m, 4H, pyridine), 7.21–7.83 (m, 4H, ph-H), 3.09 (s, 1H, -SH), 2.21 (t, 2H, -CH_2), 1.83 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 156.4, 149.4, 148.4, 147.2, 146.3, 136.6, 135.4, 133.3, 132.6, 127.1, 125.3, 125.9, 124.4, 121.2, 28.4, 21.2; MS: m/z 307 (M^+). *Anal*. Calcd for $C_{16}H_{13}N_5S$: C, 62.52; H, 4.26; N, 22.78. Found: C, 62.49; H, 4.16; N, 22.65.

4-(Indan-1-ylideneamino)-3-(2,4-dichlorophenoxymethyl)-5-mercapto-[1,2,4]triazole ***(1b).*** Yield, 69%; mp, 116–118 (°C); IR (KBr, cm^{-1}): 2778 (-SH), 1602 (>C═N), 1540, 1511, 1463 (C═C), 1099 (C-N-C); ^{1}H-NMR (DMSO-d_6) δ: 7.12–7.81 (m, 7H, ph-H), 5.01 (s, 2H, -OCH_2-), 3.02 (s, 1H, -SH), 2.32 (t, 2H, -CH_2), 1.84 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 154.4, 147.9, 140.2, 138.2, 134.4, 132.2, 131.1, 130.6, 129.4, 128.1, 127.2, 127.9, 126.4, 123.5, 121.2, 66.3, 27.2, 20.3. *Anal*. Calcd for $C_{18}H_{14}Cl_2N_4OS$: C, 53.34; H, 3.48; N, 13.82. Found: C, 53.16; H, 3.29; N, 13.75.

4-(Indan-1-ylideneamino)-3-(4-chlorophenoxymethyl)-5-mercapto-[1,2,4]triazole ***(1c).*** Yield, 70%; mp, 128–130 (°C); IR (KBr, cm^{-1}): 2753 (-SH), 1590 (>C═N), 1561, 1526, 1484 (C═C), 1103 (C-N-C); ^{1}H-NMR (DMSO-d_6) δ: 7.00–7.60 (m, 4H, ph-H), 8.0 (dd, 2H, J = 8.2, ph-H), 8.3 (dd, 2H, J = 8.3, ph-H), 4.91 (s, 2H, -OCH_2-), 2.92 (s, 1H, -SH), 2.39 (t, 2H, -CH_2), 1.79 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 152.3, 149.4, 147.2, 146.2, 133.5, 132.6, 130.4, 130.9, 128.5, 127.9, 126.3, 125.8, 125.1, 123.4, 122.4, 64.5, 26.9, 22.4. *Anal*. Calcd for $C_{18}H_{15}ClN_4OS$: C, 58.30; H, 4.08; N, 15.11. Found: C, 58.16; H, 4.01; N, 15.01.

4-(Indan-1-ylideneamino)-3-(4-methylphenoxymethyl)-5-mercapto-[1,2,4]triazole ***(1d).*** Yield, 63%; mp, 121–125 (°C); IR (KBr, cm^{-1}): 2761 (-SH), 1586 (>C═N), 1559, 1521, 1479 (C═C), 1112 (C-N-C); ^{1}H-NMR (DMSO-d_6) δ: 6.90–7.30 (m, 4H, ph-H), 7.8 (dd, 2H, J = 8.1, ph-H), 7.9 (dd, 2H, J = 8.2, ph-H), 4.81 (s, 2H, -OCH_2-), 2.81 (s, 1H, -SH), 2.26 (t, 2H, -CH_2), 1.85 (t, 2H, -CH_2), 2.13 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ: 154.4, 148.4, 147.6, 138.4, 132.5, 132.1, 129.5, 128.9, 128.1, 127.1, 125.4, 124.5, 122.6, 121.4, 65.7, 27.1, 21.3. *Anal*. Calcd for $C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N, 15.99. Found: C, 65.01; H, 5.06; N, 15.84.

4-(Indan-1-ylideneamino)-3-(2-methylphenoxymethyl)-5-mercapto-[1,2,4]triazole ***(1e).*** Yield, 57%; mp, 138–140 (°C); IR (KBr, cm^{-1}): 2763 (-SH), 1590 (>C═N), 1559, 1533, 1485 (C═C), 1083 (C-N-C); ^{1}H-NMR (DMSO-d_6) δ: 6.81–7.43 (m, 8H, ph-H), 4.89 (s, 2H, -OCH_2-), 2.78 (s, 1H, -SH), 2.24 (t, 2H, -CH_2), 1.91 (t, 2H, -CH_2), 2.24 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ: 155.5, 148.2, 146.4, 136.3, 134.4, 132.4, 131.1, 129.9, 128.5, 127.6, 126.5, 125.2, 124.8, 124.1, 123.4, 122.3, 67.2, 26.3, 22.4. *Anal*. Calcd for $C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N, 15.99. Found: C, 65.04; H, 5.10; N, 15.80.

4-(Indan-1-ylideneamino)-3-(4-nitrophenyl)-5-mercapto-[1,2,4]triazole ***(1f).*** Yield, 62%; mp, 135–136 (°C); IR (KBr, cm^{-1}): 2751 (-SH), 1586 (>C═N), 1563, 1529, 1495 (C═C), 1085 (C-N-C); ^{1}H-NMR (DMSO-d_6) δ: 6.91–7.10 (m, 4H, ph-H), 7.9 (dd, 2H, J = 8.1, ph-H), 8.0 (dd, 2H, J = 8.2, ph-H), 2.99 (s, 1H, -SH), 2.29 (t, 2H, -CH_2), 1.81 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 156.1, 147.3, 146.5, 135.2, 134.9, 133.3, 132.5, 130.4, 128.8, 126.9, 125.4, 124.5, 122.4, 121.8, 120.3, 27.1, 22.1. *Anal*. Calcd for $C_{17}H_{13}N_5O_2S$: C, 58.11; H, 3.73; N, 19.93. Found: C, 58.02; H, 3.59; N, 19.82.

4-(Indan-1-ylideneamino)-3-(4-chlorophenyl)-5-mercapto-[1,2,4]triazole ***(1g).*** Yield, 68%; mp, 193–195 (°C); IR (KBr, cm^{-1}): 2759 (-SH), 1588 (>C═N), 1569, 1523, 1484 (C═C), 1079 (C-N-C); ^{1}H-NMR (DMSO-d_6) δ: 6.96–7.44 (m, 4H, ph-H), 8.1 (dd, 2H, J = 8.2, ph-H), 8.2 (dd, 2H, J = 8.3, ph-H), 2.94 (s, 1H, -SH), 2.16 (t, 2H, -CH_2), 1.79 (t, 2H, -CH_2) ^{13}C-NMR (DMSO-d_6) δ: 156.8, 146.7, 146.1, 136.3, 135.4, 133.6, 133.1, 130.9, 127.4, 125.8, 125.1, 123.9, 123.3 121.4 120.7, 27.9, 23.2. *Anal*. Calcd for $C_{17}H_{13}ClN_4S$; C, 59.91; H, 3.84; N, 16.44. Found: C, 59.56; H, 3.67; N, 16.30.

4-(Indan-1-ylideneamino)-3-(2,4-dichlorophenyl)-5-mercapto-[1,2,4]triazole ***(1h).*** Yield, 70%; mp, 105–107 (°C); IR (KBr, cm^{-1}): 2763 (-SH), 1576 (>C═N), 1551, 1527, 1476 (C═C), 1101 (C-N-C); ^{1}H-NMR (DMSO-d_6) δ: 6.84–7.65 (m, 7H, ph-H), 2.91 (s, 1H, -SH), 2.21 (t, 2H, -CH_2), 1.79 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 157.4, 149.8, 146.8, 137.4, 136.8, 136.7, 135.8, 130.7, 128.3, 126.0, 125.9, 125.4, 124.4, 121.9, 120.0, 26.8, 23.5. *Anal*. Calcd for $C_{17}H_{12}Cl_2N_4S$: C, 54.41; H, 3.22; N, 14.93. Found: C, 54.30; H, 3.10; N, 14.69.

General method for the synthesis of 3-(3-pyridyl-5-mercapto-1,2,4-triazol-4-yl)-spiro-(indan-1′,2-thiazolidin)-4-ones 2a-2h. A mixture of 4-(indan-1-ylideneamino)-3-pyridyl-5-mercapto-[1,2,4]-triazole **1a** (1.54 g, 4.9 mmol) and mercaptoacetic acid (0.38 mL, 5.44 mmol) was heated at 40°C in polyethylene glycol (15 mL) for 5–6 h. The reaction mixture was poured into ice cold water and washed with sodium bicarbonate solution. The crude product thus obtained was filtered, dried, and recrystallized from dioxan : water (1.17 g, 61%). Similarly, the other compounds were prepared.

3-[3-Pyridyl-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-one ***(2a).*** Yield, 63%; mp, 240–242 (°C); IR (KBr, cm^{-1}): 2750 (-SH), 1708 (>C═O), 1555, 1511, 1456 (C═C), 703 (C-S-C); ^{1}H-NMR (DMSO-d_6) δ: 8.01–8.81 (m, 4H, pyridine), 7.21–7.62 (m, 4H, ph-H), 3.91 (s, 2H, -CH_2-CO-), 3.01 (s, 1H, -SH), 2.23 (t, 2H, -CH_2), 1.79 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 186.4, 158.6, 150.3, 148.1, 145.2, 138.3, 129.4,

128.2, 127.9, 126.5, 125.9, 124.6, 123.4, 121.1, 68.7, 66.5, 35.2, 27.3; MS: m/z 381 (M^+). *Anal.* Calcd for $C_{18}H_{15}N_5OS_2$: C, 56.67; H, 3.96; N, 18.36; Found: C, 56.45; H, 3.81; N, 18.25.

3-[3-(2,4-Dichlorophenoxymethyl)-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-one ***(2b)***. Yield, 62%; mp, 128–130 (°C); IR (KBr, cm^{-1}): 2761 (-SH), 1710 (>C═O), 1559, 1523, 1461 (C═C), 697 (C-S-C);^{1}H-NMR (DMSO-d_6) δ: 6.91–7.51 (m, 7H, ph-H), 4.84 (s, 2H, -OCH_2-), 3.63 (s, 2H, -CH_2-CO-), 2.91 (s, 1H, -SH), 2.19 (t, 2H, -CH_2), 1.74 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 189.1, 151.9, 148.4, 141.2 138.6, 133.4, 130.3, 128.8, 128.1, 127.5, 126.4, 125.1, 123.5, 121.8, 121.2, 68.1, 66.2, 38.2, 34.2, 24.3. *Anal*. Calcd for $C_{20}H_{16}N_4O_2S_2Cl_2$: C, 50.11; H, 3.36; N, 11.69. Found: C, 49.90; H, 3.26; N, 11.50.

3-[3-(4-Chlorophenoxymethyl)-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-one ***(2c)***. Yield, 67%; mp, 141–142 (°C); IR (KBr, cm^{-1}): 2764 (-SH), 1714 (>C═O), 1563, 1510, 1483 (C═C), 701 (C-S-C); ^{1}H-NMR (DMSO-d_6) δ: 6.94–7.69 (m, 4H, ph-H), 8.1 (dd, 2H, J = 8.2, ph-H), 8.3 (dd, 2H, J = 8.3, ph-H), 4.69 (s, 2H, -OCH_2-), 3.72 (s, 2H, -CH_2-CO-), 2.98 (s, 1H, -SH), 2.23 (t, 2H, -CH_2), 1.78 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 184.3, 148.3, 146.2, 140.6, 136.4, 135.8, 132.6, 130.9, 126.9, 126.2, 125.2, 121.3, 121.0, 69.8, 66.2, 35.3, 35.1, 24.4. *Anal*. Calcd for $C_{20}H_{17}N_4O_2S_2Cl$: C, 53.99; H, 3.85; N, 12.59. Found: C, 53.80; H, 3.79; N, 12.46.

3-[3-(4-Methylphenoxymethyl)-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-one ***(2d)***. Yield, 61%; mp, 143–145 (°C); IR (KBr, cm^{-1}): 2770 (-SH), 1701 (>C═O), 1570, 1523, 1469 (C═C), 698 (C-S-C); ^{1}H-NMR (DMSO-d_6) δ: 6.69–7.44 (m, 4H, ph-H), 7.8 (dd, 2H, J = 8.0, ph-H), 7.9 (dd, 2H, J = 81, ph-H), 4.64 (s, 2H, -OCH_2-), 3.81 (s, 2H, -CH_2-CO-), 2.99 (s, 1H, -SH), 2.28 (t, 2H, -CH_2), 1.81 (t, 2H, -CH_2), 2.19 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ: 185.2, 141.9, 139.2, 138.8, 131.2, 130.4, 129.6, 128.7, 124.3, 123.4, 122.8, 122.2, 120.4, 71.2, 64.1, 37.4, 36.1, 25.4, 23.1. *Anal*. Calcd for $C_{21}H_{20}N_4O_2S_2$: C, 59.41; H, 4.75; N, 13.20. Found: C, 59.34; H, 4.62; N, 13.11.

3-[3-(2-Methylphenoxymethyl)-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-one ***(2e)***. Yield, 54%; mp, 180–183 (°C); IR (KBr, cm^{-1}): 2778 (-SH), 1709 (>C═O), 1564, 1536, 1475 (C═C), 691 (C-S-C); ^{1}H-NMR (DMSO-d_6) δ: 6.68–7.61 (m, 8H, ph-H), 4.62 (s, 2H, -OCH_2-) 3.90 (s, 2H, -CH_2-CO-), 2.96 (s, 1H, -SH), 2.21 (t, 2H, -CH_2), 1.79 (t, 2H, -CH_2), 2.21 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) δ: 190.1, 149.4, 146.1, 144.2, 139.4, 133.2, 130.9, 128.6, 127.9, 127.2, 126.8, 125.4, 124.7, 124.0, 123.7, 68.5, 62.1, 34.6, 31.5, 24.8, 23.2. *Anal*. Calcd For $C_{21}H_{20}N_4O_2S_2$: C, 59.41; H, 4.75; N, 13.20. Found: C, 59.32; H, 4.62; N, 13.12.

3-[3-(4-Nitrophenyl)-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-one ***(2f)***. Yield, 61%; mp, 158–160 (°C); IR (KBr, cm^{-1}): 2758 (-SH), 1703 (>C═O), 1570, 1531, 1483 (C═C), 699 (C-S-C); ^{1}H-NMR (DMSO-d_6) δ: 6.86–7.2 (m, 4H, ph-H), 7.9 (dd, 2H, J = 8.2, ph-H), 8.0 (dd, 2H, J = 8.1, ph-H), 3.84 (s, 2H, -CH_2-CO-), 2.98 (s, 1H, -SH), 2.21 (t, 2H, -CH_2), 1.79 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 187.3, 150.3, 145.6, 140.2, 131.8, 129.7, 128.4, 127.4, 126.2, 125.2, 124.8, 121.6, 120.3, 68.2, 35.7, 32.6, 24.9. *Anal*. Calcd for $C_{19}H_{15}N_5O_3S_2$: C, 53.63; H, 3.55; N, 16.46; Found: C, 53.46; H, 3.49; N, 16.20.

3-[3-(4-Chlorophenyl)-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-one ***(2g)***. Yield, 67%; mp, 213–215 (°C); IR (KBr, cm^{-1}): 2769 (-SH), 1710 (>C═O), 1584, 1539, 1475 (C═C), 693 (C-S-C); ^{1}H-NMR (DMSO-d_6) δ: 6.95–7.44 (m, 4H, ph-H), 8.2 (dd, 2H, J = 8.3, ph-H), 8.3 (dd, 2H, J = 8.4, ph-H), 3.79 (s, 2H, -CH_2-CO-), 3.01 (s, 1H, -SH), 2.29 (t, 2H, -CH_2), 1.80 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 189.2, 149.4, 144.1, 140.4, 132.4, 128.0, 127.4, 126.2, 125.9, 125.1, 124.6, 121.3, 120.1, 69.1, 36.8, 31.5, 23.8. *Anal*. Calcd for $C_{19}H_{15}ClN_4OS_2$: C, 55.00; H, 3.64; N, 13.50. Found: C, 54.81; H, 3.51; N, 13.26.

3-[3-(2,4-Dichlorophenyl)-5-mercapto-1,2,4-triazol-4-yl]-spiro-(indan-1′,2-thiazolidin)-4-one ***(2h)***. Yield, 64%; mp, 123–125 (°C); IR (KBr, cm^{-1}): 2774 (-SH), 1714 (>C═O), 1585, 1536, 1461 (C═C), 689 (C-S-C); ^{1}H-NMR (DMSO-d_6) δ: 6.68–7.63 (m, 7H, ph-H), 3.70 (s, 2H, -CH_2-CO-), 2.94 (s, 1H, -SH), 2.24 (t, 2H, -CH_2), 1.76 (t, 2H, -CH_2); ^{13}C-NMR (DMSO-d_6) δ: 190.2, 149.9, 148.4, 146.2, 143.2, 138.4, 135.2, 131.8, 131.2, 128.9, 128.4, 127.2, 123.1, 122.1, 120.3, 68.1, 38.5, 36.4, 26.1. *Anal*. Calcd for $C_{19}H_{14}Cl_2N_4OS_2$: C, 50.78; H, 3.14; N, 12.47. Found: C, 50.60; H, 3.10; N, 12.32.

Biological screening

Antibacterial screening. Preliminary experiments were carried out to determine the antibacterial activities of spirothiazolidinone derivatives *in vitro* against (i) Gram-positive bacteria, *S. pneumoniae* and *B. subtilis*, and (ii) Gram-negative bacteria, *E. coli* and *P. aeruginosa*, by disk diffusion method. The bacterial strains were subcultured in broth agar and incubated for 18 h at 37°C, and then freshly prepared bacterial cells were spread onto nutrient agar plate in a laminar flow cabinet. Sterilized paper disks (6.0 mm in diameter) were placed on the nutrient agar plates. Five milligrams of each test compounds were dissolved in 1 mL of DMSO separately to prepare stock solution. From stock solution, different concentrations 50, 25, 12.5, 6.25, and 3.12 μg/mL of each compound were prepared. Thus, proper amounts of the different concentrations of compounds were pipetted on the blank disks, which were placed on the plates. The plates were incubated at 37°C for 24 h. The MICs, the lowest concentration (μg/mL) of the test compound that resulted no visible growth on the plate, were recorded in Table 1. DMSO was used as a solvent control to ensure that the solvent had no effect on bacterial growth. Ciprofloxacin was designated in our experiment as a control drug.

Antifungal screening. Titled compounds were screened for their antifungal activity against *C. albicans*, *A. fumigatus*, and *A. flavus* (recultured) in DMSO by serial plate dilution method. Test compound (5 mg) were dissolved in 1 mL of DMSO, and solution was diluted with water (9 mL). Further progressive dilutions with melted Mueller–Hinton agar were performed to obtain required concentrations of 50, 25, 12.5, 6.25, and 3.12 μg/mL. Petri dishes were inoculated with 1.5×10^{-4} colony forming units (CFU) and incubated at 37°C for 26 h. The MICs in μg/mL were noted. To ensure that solvent had no effect on fungal growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. Fluconazole was used as a standard drug.

Antitubercular activity. Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* $H_{37}Rv$ were performed by agar microdilution method where serial twofold dilutions of each test compound were added into 7H10 agar, and *M. tuberculosis* $H_{37}Rv$ was used as test organism. MIC is the concentration of the compound that completely inhibits the growth and colony forming ability of *M. tuberculosis*. In a 24-well plate, 3 mL Middlebrook 7H11 agar medium with OADC supplement is dispensed in each well. The test compound is added to the Middlebrook medium agar before in duplicate so that final concentration of test compound in each well is 12.5, 6.25, 3.12, and 1.56 μg/mL, respectively. The known CFU of $H_{37}Rv$ culture was dispensed on top of agar in each well in negative pressure biosafety hood. The plates are then incubated at 37°C/5% CO_2 incubator. The concentration at which complete inhibition of colonies was observed was taken as MIC of test drug. Isoniazid was used as a standard drug.

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REFERENCES AND NOTES

[1] Gold, H. C.; Moellering Jr., R. C. N. Eng J Med 1996, 335, 1445.
[2] Davis, J. Science 1994, 264, 357.
[3] Avilffe, A. G. Clin Infect Dis 1997, 24, 874.
[4] Kontoiannis, D. D.; Somonis, G. J. Hosp Infec 2001, 53, 1.
[5] Garber, G. Drugs 2001, 61, 1.
[6] Hrib, N.; Jurcak, J. J Med Chem 1992, 35, 2712.
[7] Deryk, R. B.; Zimenkocsky, B. S. Current Org Chem B 2004, 8, 1547.
[8] Biradar, J. S.; Manjunath S. Y. Ind J Chem 2004, 43B, 389.
[9] Strumvoll, M.; Haring, H. U. Ann Med 2002, 34, 217.
[10] Unanagst, P. C.; Connor, D. T.; Catenko, W. A.; Sorenson, R. J.; Sircar, J. C.; Wright, C. D.; Schrier, D. J.; Dyer, R. D. Bioorg Med Chem Lett 1993, 3, 1729.
[11] Delima, M. C. A.; Costa, D. L. B.; Goes, A. J. S.; Galdino, S. L.; Pitta, I. R.; Luu-Duc, C. Pharmazie 1992, 47, 182.
[12] Andreani, A.; Rambaldi, M.; Locatelli, A.; Leoni, R.; Bossa, M.; Chiericozzi, I.; Galatulas, G.; Salvatore, A. Eur J Med Chem 1993, 28, 825.
[13] El-Kanzi, N. A. A.; Soleiman, H. A.; Khalafallah, A. K. Phosphorus, Sulfur and Silicon 2007, 182, 1459.
[14] Bhambi, D.; Salvi, V. K.; Jat, J. L.; Ojha, S.; Talesara, G. L. J Sulfur Chem 2007, 28, 155.
[15] Lesyk, R.; Vladzimirska, O.; Zimenkovsky, B.; Golota, S.; Nektegagayev, I.; Cherpark, O.; Leb'yak, M.; Kozak, O. Boll Chim Farm 2002, 144, 197.
[16] Desai, N. C.; Shukla, H. K.; Thaker, K. A. J. Indian Chem Soc1984, 62, 242.
[17] Babaoglu, K.; Page, M. A.; Jones V. C.; McNeil, M. R.; Dong, C.; Naismith, J. H.; Lee, R. E. Bioorg Med Chem Lett 2003, 13, 3227.
[18] Unangst, P. C.; Shurum, G. P.; Connor, D. T.; Dyer, R. D.; Schrier, D. J. J Med Chem 1992, 35, 3691.
[19] Holla, B. S.; Akberali, P. M.; Shivananda, M. K. Farmaco 2001, 56, 919.
[20] Wujec, M.; Pitucha, M.; Dobosz, M.; Kosikowska, U.; Malm, A. Acta Pharm 2004, 54, 251.
[21] Holla, B. S.; Sarojini, B. K.; Akberali, P. M.; Kumari, N. S. Eur J Med Chem 2006, 41, 657.
[22] Vamvakides, A. Pharm Fr 1990, 48, 154.
[23] Ona, M.; Aoki, K. Jpn. Kokai 116,762, 1989; Chem Abstr 1989, 111, 778489.
[24] Skiles, J. W.; Menil, D. Tetrahedron Lett 1990, 31, 7277.
[25] Khan, M. H.; Tiwari, S.; Begum, K. Nizamuddin Indian J Chem 1998, 37B, 1075.
[26] Joshi, K. C.; Jain, R.; Chand, P. Heterocycles 1985, 23, 957.
[27] Alauddin, S.; Nizamuddin Indian J Chem 2002, 41B, 1314.
[28] Nizamuddin; Singh, A. Indian J Chem 2004, 43B, 901.
[29] Singh, A.; Nizamuddin J. Indian Chem Soc 2005, 82, 727.
[30] Pandey, S. K.; Nizamuddin Arch Pharm Chem Life Sci 2008, 341, 418.
[31] Pandey, S. K.; Singh, A.; Singh, A.; Nizamuddin Eur J Med Chem 2009, 44, 1188.
[32] Yao, S. W.; Lopes, V. H. C.; Fernandez, F.; Mera, X. G.; Morales, M.; Borges, J. E. R.; Cordeiro, S. D. N. M. Biorg Med Chem 2003, 11, 4999.
[33] Rich, S. Fungicidal Chemistry in Plant Pathology; Academic Press Ltd.: New York, 1960, 2, 588.
[34] Eyring, H. Science 1966, 154, 1609.
[35] Jain, S. L.; Singhal, S.; Sain, B. Green Chem 2007, 9, 740.
[36] Wang, X.; Gong, H.; Quan, Z.; Li, L.; Ye, H. Synthetic Commun 2011, 21, 3251.
[37] (a)Lui, X. H.; Ludley, P. Tetrahedron 2006, 62, 11039; Heldebrant, D.; Jessop, P. G. J. Am Chem Soc 2003, 125, 5600; (b)Wang, X.; Quan, Z.; Zhang, Z. Tetrahedron 2007, 63, 8227.
[38] Bauer, A. W.; Kirby, W. M.; Sherris J. C.; Turck, M. Am J Clin Pathol 1966, 45, 493.
[39] Khan, Z. K. In vitro and vivo screening techniques for bioactivity screening and evaluation. Proceeding Int. Workshop UNIDO-CDRI, 1997, pp. 210.
[40] Saito, H.; Tomioka, H.; Sato, K.; Emori, M.; Yamane, T.; Yamashita, K.; Hosol, K. Hidaka, T. Antimicrob Agents Chemother 1991, 35, 542.
[41] Tomayo, M. L. Bull. Soc. Chim. France 20, 1961; Chem Abstr 1962, 57, 483d.