Synthesis and Antimicrobial Activity of Some 5-Substituted-3-phenyl-N_β-(Substituted-2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-1*H*-indole-2-carboxyhydrazide

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Ethyl 3-oxo-3-{2-[(5-substituted-3-phenyl-1*H*-indol-2-yl)carbonyl]hydrazinyl}propanoates 5a—b were synthesized according to the literature method. These on further reaction with substituted-2-hydroxy-3-formylquinolines 3a—e yielded 5-substituted-N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazides 6a—j. Structures of the all the newly synthesized compounds were confirmed by spectral data. All these compounds have been screened for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilus*, antifungal activity against *Aspergillus niger* and *Candida albicans* and antituberculosis activity against *Mycobacterium tuberculosis* (H37R$_v$).

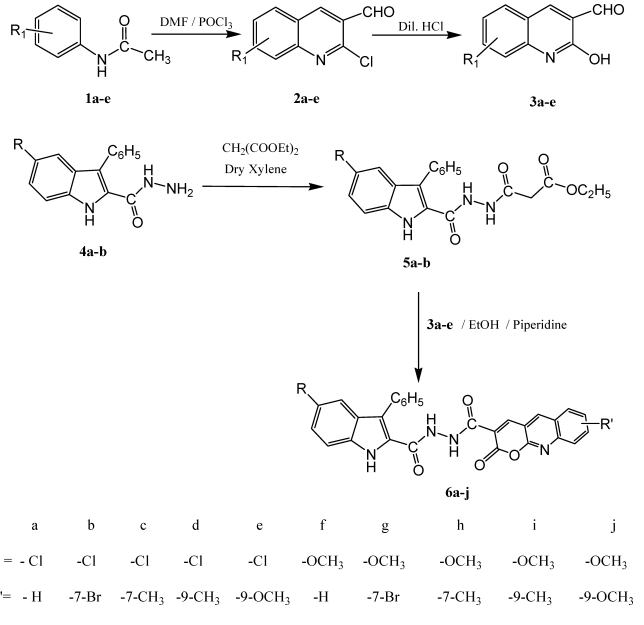
Key words indole; quinoline; pyrano-quinoline-2-one; antimicrobial activity

Tuberculosis is an infection caused by *Mycobacterium tuberculosis*, which commonly affects the respiratory tract, *i.e.* lungs.[1] It is termed as "a global health emergency" by World Health Organization (WHO) in 1993 as it affects 1.7 billion people per year that is equal to one-third of the entire world population. The first line of drugs used in the treatment of tuberculosis (TB) is a combination of isoniazid, rifamycin, pyrazinamide and ethambutal. The high concentration of lipids in the cell wall of *M. tuberculosis* has been attributed to its resistant to antibiotics. Thus the increasing clinical importance of tuberculosis has lent additional urgency to researchers to identify new effective antimycobacterial compounds.[2]

Heterocycles bearing nitrogen, sulphur and oxygen atoms in their structure constitute the core structure of a number of biologically interesting compounds. Many indole derivatives reported in the literature are known to possess varied biological activities *viz.*, antimalarial activity,[3] antituberculosis activity[4] and COX-2 inhibitors.[5] Quinolines derivatives have attracted the attention of the chemists because of their presence in many natural products possessing significant biological activities.[6—10] Many indolo[2,3-*c*]isoquinolines reported from our laboratory have been found to possess bactericidal and fungicidal[11—13] activities. Earlier we have reported the synthesis of 3,5-disubstituted-N_β-(2-oxobenzopyran-3-carbonyl)-1*H*-indole-2-carbohydrazide[14] by making use of ethyl 3-oxo-3-{2-[(5-substituted-3-phenyl-1*H*-indol-2-yl)carbonyl]hydrazinyl}propanoates **5a**, **b**, which in turn were prepared by the reaction of 5-substituted-3-phenyl-1*H*-indole-2-carbohydrazides **4a**, **b** and diethylmalonate. In view of these findings and in continuation of our research work on indoles,[15—18] we hereby report the synthesis and antimicrobial activity of some 5-substituted-N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazides **6a**—**j** having indole and pyranoquinoline moieties in their structure with the hope getting compound with more potent antimicrobial and antituberculosis activity by making use of ethyl 3-oxo-3-{2-[(5-substituted-3-phenyl-1*H*-indol-2-yl)carbonyl]hydrazinyl}propanoates **5a**, **b** and substituted-2-hydroxy-3-formylquinolines **3a**—**e** as starting materials wherein substituted-2*H*-pyrano[2,3-*b*]quinoline moiety attached to β-nitrogen of 5-substituted-3-phenyl-1*H*-indole-2-carbohydrazide at its 3-positon *via* carbonyl group (Chart 1). These compounds are novel and hitherto unknown.

Results and Discussion

Compounds **5a**, **b** were prepared according to the reported method.[15] These compounds **5a**, **b** when reacted with substituted-2-hydroxy-3-formyl-quinolines **3a**—**e** in the presence of catalytic amount of piperdine in ethanol under refluxed conditions for 5 h afforded 5-substituted-N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazides **6a**—**j** in a good yield. Compound **6a** in its IR spectrum showed absorption bands at 1149, 1597, 1668, 1695, 1733, 3065, 3201 and 3386 cm^{-1} due to C–O–C, C=N, C=O/C=O/C=O and NH/NH/NH functions respectively. Three singlets and a multiplet, observed at 9.42, 9.71, 10.20 and 7.14—7.92 δ in the ^{1}H-NMR spectrum of compound **6a** were due to one proton of indole NH and two pro-



	a	b	c	d	e	f	g	h	i	j
R =	- Cl	-Cl	-Cl	-Cl	-Cl	-OCH$_3$	-OCH$_3$	-OCH$_3$	-OCH$_3$	-OCH$_3$
R'=	- H	-7-Br	-7-CH$_3$	-9-CH$_3$	-9-OCH$_3$	-H	-7-Br	-7-CH$_3$	-9-CH$_3$	-9-OCH$_3$

Chart 1

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tons of two CONH functions and fourteen aromatic protons respectively. Mass spectrum of compound **6a** exhibited molecular ion peak $M^{+\cdot}$ at 508, 510 (33%, 12%), which corresponds to its molecular weight. This molecular ion due to the loss of 2*H*-pyrano[2,3-*b*]quinolin-2-one molecule gave a fragment ion recorded at *m*/*z* 311, 313 (48%, 19%), which on simultaneous expulsion of CO and N_2 molecule gave another fragment ion recorded at *m*/*z* 255, 257 (100%, 31%) which is also a base peak. This fragment ion on simultaneous loss of formyl and hydrogen radicals followed by the loss of chloride radical gave fragment ions recorded at *m*/*z* 225, 227 (24%, 9%) and 190 (36%) respectively (Chart 2). The IR, ^{1}H-NMR and mass spectral fragmentation data of compound **6a** are in consistancy with its structure and prove the formation of compound **6a** from compound **5a** on reaction with 2-hydroxy-3-formylquinoline **3a**.

Antimicrobial Activity The results showed that the compounds **6a**, **6b**, **6f** and **6g** showed good activity and compounds **6c**, **6d**, **6e** and **6j** exhibited moderate activity against *Staphylococcus aureus* when compared to that of standard drug Gentamycin at the same concentration as that of test drugs. Compounds **6a**, **6b**, **6d**, **6f** and **6g** showed good activity and compounds **6c**, **6e**, **6h**, **6i** and **6j** exhibited moderate activity when compared to that of standard drug Gentamycin at the same concentration as that of test drugs against *Escherichia coli*. Compounds **6a**, **6b**, **6f** and **6g** showed good activity and compounds **6d**, **6e**, **6i** and **6j** exhibited moderate activity against *Bacillus subtilus* when compared to that of standard drug Gentamycin at the same concentration as that of test drugs. Compounds **6a**, **6b**, **6f**, **6g** and **6i** showed good activity and compounds **6c**, **6d**, **6e**, **6h**, and **6j** exhibited moderate activity when compared to that of standard drug Nystatin at the same concentration as that of test drugs against *Aspergillus niger*. Compounds **6a**, **6b**, **6f** and **6g** showed good activity and compounds **6c**, **6d**, **6e**, **6h**, **6i** and **6j** exhibited moderate activity when compared to that of standard drug Nystatin at the same concentration as that of test drugs against *Candida albicans*. Rest of the compounds showed less activity against all the microorganisms tested. Under these conditions control *N*,*N*-dimethylformamide (DMF) did not show any antibacterial and antifungal activity.

Antituberculosis Activity The results showed that the compounds **6a**, **6b** and **6g** inhibited the growth of *M. tuberculosis* at concentration 12.5 μg/ml. Compounds **6d** and **6f** exhibited moderate activity when compared to standard drug streptomycin against *M. tuberculosis* which showed minimum inhibitory concentration at 25 μg/ml. Rest of the compounds showed activity at higher concentration when compared to standard drug Streptomycin against the microorganism tested. Under these conditions standard drug streptomycin was sensitive at concentration 6.25 μg/ml and control DMF did not show any antituberculosis activity.

Conclusion

The synthesis of the target novel compounds 5-substituted-

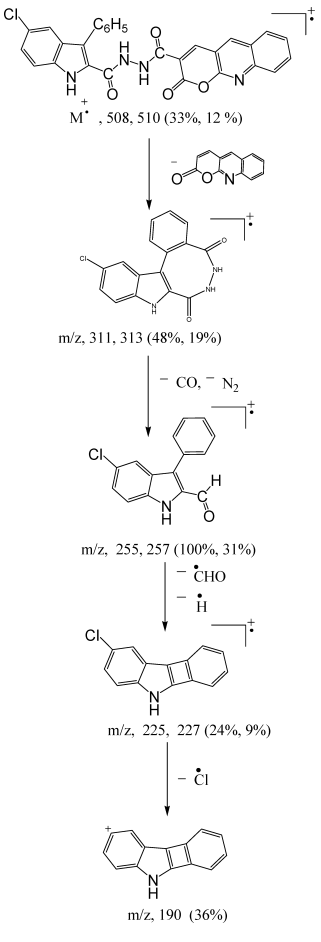

Chart 2

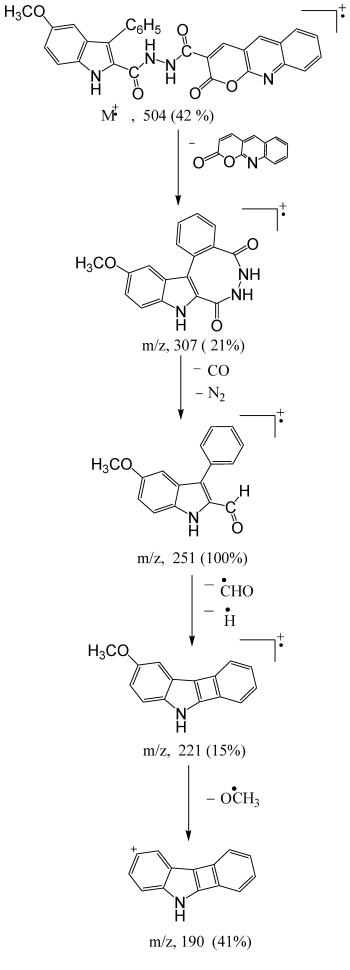

Chart 3

N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide **6a**—**j** was achieved according to the steps indicated in Chart 1. These reactions are simple, easily carried under normal reaction conditions and these systems are novel and hitherto unknown.

All the newly synthesized 5-substituted-N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide **6a**—**j** compounds tested for their antibacterial activity against *S. aureus*, *E. coli* and *B. substilus* and antifungal activity against *A. niger* and *A. flavous*. Compounds **6a**, **6b**, **6f** and **6g** showed good activity against the above microorganisms tested when compared with those of standards Gentamycin and Nystatin which were used at the same concentration (1000 μg/ml in DMF) as that of test drugs.

All the newly synthesized compounds 5-substituted-N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazides **6a**—**j** were tested for their antituberculosis activity against *M. tuberculosis*. Compounds **6a**, **6b**, **6f** and **6g** showed good activity compared with standard drug streptomycin.

Experimental

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr discs (ν_{max} in cm^{-1}) on Perkin-Elmer FT-IR (Spectrum ONE) spectrophotometer, ^{1}H-NMR spectra on a Bruker AMX (400 MHz) spectrophotometer using DMSO-d_6 as solvent using TMS as an internal standard (chemical shifts in δ) and mass spectra on a mass spectrophotometer JOEL sx-102 (FAB) instrument. Compounds were checked for their purity by TLC on silica gel G plates and spots were located by iodine vapours.

The starting materials ethyl 3-oxo-3-{2-[(5-substituted-3-phenyl-1*H*-indol-2-yl)carbonyl]hydrazinyl}propanoates[15] **5a**, **b** and substituted-2-hydroxy-3-formylquinolines[10,11] **3a**—**e** were papered according to reported methods.

Synthesis of Substituted-2-chloro-3-formylquinolines 2a—e Dimethyl formamide (0.006 mol) was cooled to 0 °C in a flask equipped with a drying tube and phosphorousoxychloride (0.006 mol) was added dropwise with stirring. To this solution acetanilide (**1a**—**e**) (0.001 mol) was added in small portions and after 5 min the reaction mixture was heated for 16 h on boiling water bath. The reaction mixture was poured into ice water and stirred for 30 min. The solid separated was filtered, dried and recrystallized from ethyl acetate to get substituted-2-chloro-3-formyl quinolines **2a**—**e** in good yield.

Synthesis of Substituted-2-hydroxy-3-formylquinolines 3a—e A mixture of 2-chloro-3-formylquinolines **2a**—**e** (0.001 mol) and aqueous hydrochloric acid 3.5 ml (4 N) was heated under reflux conditions for 1 h and then allowed to cool to room temperature. The reaction mixture was poured on to crushed ice and the solid separated filtered, washed with water, dried and recrystallized from aqueous acetic acid to get substituted-2-hydroxy-3-formylquinolines **3a**—**e** in good yield.

Synthesis of 5-Substituted-N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazides 6a—j A mixture of compounds ethyl 3-oxo-3-{2-[(5-substituted-3-phenyl-1*H*-indol-2-yl)carbonyl]hydrazinyl}propanoates **5a**, **b** (0.001 mol) and various substituted-2-hydroxy-3-formylquinolines **3a**—**e** (0.001 mol) in ethanol (10 ml) was refluxed for 5 h in the presence of catalytic amount of piperdine. Excess of ethanol was removed by distillation. Crystalline residue obtained was filtered, washed with little amount of ethanol, dried and crystallized from suitable solvent to afford 5-substituted-N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazides **6a**—**j** in good yield. The compounds **6a**, **6b**, **6c**, **6d**, **6e** and **6g** were recrystallized from ethanol and the compounds **6f**, **6h**, **6i** and **6j** were recrystallized from isopropyl alcohol.

5-Chloro-N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6a**): Colourless crystals in 71% yield, mp 212 °C: IR (KBr) in cm^{-1}: 1149 (C–O–C), 1597 (C=N), 1668, 1695, 1733 (CO/CO/CO), 3065, 3201, 3386 (NH/NH/NH). ^{1}H-NMR in δ: 7.14—7.92 (m, 14H, ArH), 9.42 (s, 1H, indole NH), 9.71 (s, 1H, CONH), 10.20 (s, 1H, CONH). FAB-MS *m*/*z* (in %): 508, 510 (33%, 12%), 311, 313 (48%, 19%), 255, 257 (100%, 31%), 225, 227 (24%, 9%), 190 (36%) (Chart 2). *Anal.* Calcd for $C_{28}H_{17}N_4O_4Cl$: C, 66.08; H, 3.34; N, 11.01; Found: C, 65.91; H, 3.42; N, 10.89.

5-Chloro-N_β-(7-bromo-2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6b**): Pale yellow crystals in 69% yield, mp 185 °C: IR (KBr) cm^{-1}: 1158 (C–O–C), 1593 (C=N), 1663, 1701, 1731 (C=O/C=O/C=O), 3050, 3211, 3305 (NH/NH/NH).^{1}H-NMR in δ: 6.80—7.84 (m, 13H, ArH), 9.58 (s, 1H, indole NH), 9.95 (s, 1H, CONH), 10.19 (s, 1H, CONH). *Anal.* Calcd for $C_{28}H_{16}N_4O_4ClBr$: C, 57.19; H, 2.72; N, 9.53; Found: C, 56.98; H, 2.58; N, 9.34.

5-Chloro-N_β-(7-methyl-2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6c**): Colourless crystals in 75% yield, mp 145 °C: IR (KBr) cm^{-1}: 1154 (C–O–C), 1564 (C=N), 1667, 1689, 1713 (CO/CO/CO), 3060, 3195, 3281 (NH/NH/NH). ^{1}H-NMR in δ: 1.74 (s, 3H, CH_3), 7.12—7.80 (m, 13H, ArH), 9.48 (s, 1H, indole NH), 9.84 (s, 1H, CONH), 10.28 (s, 1H, CONH). *Anal.* Calcd for $C_{29}H_{19}N_4O_4Cl$: C, 66.60; H, 3.64; N, 10.71; Found: C, 66.48; H, 3.52; N, 10.55.

5-Chloro-N_β-(9-methyl-2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6d**): Colourless crystals in 64% yield, mp 191 °C: IR (KBr) cm^{-1}: 1162 (C–O–C), 1579 (C=N), 1668, 1695, 1723 (CO/CO/CO), 3061, 3187, 3258 (NH/NH/NH). ^{1}H-NMR in δ: 1.98 (s, 3H, CH_3), 7.21—7.91 (m, 13H, ArH), 9.18 (s, 1H, indole NH), 9.70 (s, 1H, CONH), 10.18 (s, 1H, CONH). *Anal.* Calcd for $C_{29}H_{19}N_4O_4Cl$: C, 66.60; H, 3.64; N, 10.71; Found: C, 66.41; H, 3.48; N, 10.61.

5-Chloro-N_β-(9-methoxy-2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6e**): Brown crystals in 71% yield, mp 231 °C: IR (KBr) cm^{-1}: 1162 (C–O–C), 1578 (C=N), 1671, 1692, 1719 (CO/CO/CO), 3081, 3188, 3280 (NH/NH/NH). ^{1}H-NMR in δ: 2.28 (s, 3H, $-OCH_3$), 7.32—7.98 (m, 13H, ArH), 9.61 (s, 1H, indole NH), 10.0 (s, 1H, CONH), 10.36 (s, 1H, CONH). *Anal.* Calcd for $C_{29}H_{19}N_4O_5Cl$: C, 64.62; H, 3.53; N, 10.40; Found: C, 64.40; H, 3.35; N, 10.25.

5-Methoxy-N_β-(2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6f**): Pale yellow crystals in 69% yield, mp 189 °C: IR (KBr) cm^{-1}: 1166 (C–O–C), 1578 (C=N), 1681, 1698, 1719 (CO/CO/CO), 3083, 3105, 3187 (NH/NH/NH). ^{1}H-NMR in δ: 2.15 (s, 3H, $-OCH_3$), 7.01—7.78 (m, 14H, ArH), 9.65 (s, 1H, indole NH), 9.91 (s, 1H, CONH), 10.28 (s, 1H, CONH). FAB-MS *m*/*z* (in %): 504 (42%), 307 (21%), 251 (100%), 221(15%), 190 (41%) (Chart 3). *Anal.* Calcd for $C_{29}H_{20}N_4O_5$: C, 69.05; H, 3.97; N, 11.11; Found: C, 68.80; H, 3.75; N, 11.05.

5-Methoxy-N_β-(7-bromo-2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6g**): Brown crystals in 72% yield, mp 225 °C: IR (KBr) cm^{-1}: 1154 (C–O–C), 1599 (C=N), 1678, 1698, 1728 (CO/CO/CO), 3058, 3108, 3281 (NH/NH/NH). ^{1}H-NMR in δ: 2.18 (s, 3H, $-OCH_3$), 6.91—7.68 (m, 13H, ArH), 9.80 (s, 1H, indole NH), 10.28 (s, 1H, CONH), 10.57 (s, 1H, CONH). *Anal.* Calcd for $C_{29}H_{19}N_4O_5Br$: C, 59.69; H, 3.26; N, 9.60; Found: C, 59.48; H, 3.14; N, 9.39.

5-Methoxy-N_β-(7-methyl-2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6h**): Colourless crystals in 64% yield, mp 251 °C: IR (KBr) cm^{-1}: 1162 (C–O–C), 1580 (C=N), 1661, 1680, 1723 (CO/CO/CO), 3061, 3188, 3251 (NH/NH/NH). ^{1}H-NMR in δ: 1.71 (s, 3H, CH_3), 2.20 (s, 3H, $-OCH_3$), 7.14—7.78 (m, 13H, ArH), 9.76 (s, 1H, indole NH), 10.19 (s, 1H, CONH), 10.56 (s, 1H, CONH). *Anal.* Calcd for $C_{30}H_{22}N_4O_5$: C, 69.50; H, 4.25; N, 10.81; Found: C, 69.36; H, 4.15; N, 10.60.

5-Methoxy-N_β-(9-methyl-2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6i**): Colourless crystals in 71% yield, mp 165 °C: IR (KBr) cm^{-1}: 1166 (C–O–C), 1578 (C=N), 1668, 1685, 1719 (CO/CO/CO), 3083, 3187, 3248 (NH/NH/NH).^{1}H-NMR in δ: 1.74 (s, 3H, CH_3), 2.18 (s, 3H, $-OCH_3$), 7.10—7.79 (m, 13H, ArH), 9.36 (s, 1H, indole NH), 9.78 (s, 1H, CONH), 10.08 (s, 1H, CONH). *Anal.* Calcd for $C_{30}H_{22}N_4O_5$: C, 69.50; H, 4.25; N, 10.81; Found: C, 69.70; H, 4.05; N, 10.58.

5-Methoxy-N_β-(9-methoxy-2-oxo-2*H*-pyrano[2,3-*b*]quinoline-3-carbonyl)-3-phenyl-1*H*-indole-2-carbohydrazide (**6j**): Colourless crystals in 69% yield, mp 158 °C: IR (KBr) cm^{-1}: 1162 (C–O–C), 1578 (C=N), 1661, 1678, 1723 (CO/CO/CO), 3057, 3186, 3281 (NH/NH/NH).^{1}H-NMR in δ: 2.19 (s, 3H, $-OCH_3$), 2.41 (s, 3H, $-OCH_3$), 7.21—7.79 (m, 13H, ArH), 10.00 (s, 1H, indole NH), 10.25 (s, 1H, CONH), 10.58 (s, 1H, CONH). *Anal.* Calcd for $C_{30}H_{22}N_4O_6$: C, 67.42; H, 4.12; N, 10.49; Found: C, 67.31; H, 4.19; N, 10.68.

Antibacterial and Antifungal Testing Method The *in vitro* biological screening of the compounds **6a**—**j** was undertaken against the bacteria *S. aureus*, *E. coli* and *B. subtilus*, fungi *A. niger* and *C. albicans* by cup-plate method[14—16] using nutrient agar as medium. Then holes of 6 mm diameter were punched carefully using a sterile cork borer and these were filled with test solutions (1000 μg/ml in DMF) and DMF used as control. The plates

Table 1. Antimicrobial Activity of Synthesized Compounds

Compound	Zone of inhibition in mm[a] (activity index)					Antituberculosis activity in MIC (μg/ml)
	Antibacterial activity (1000 μg/ml)			Antifungal activity (1000 μg/ml)		
	S. aureus	*E. coli*	*B. subtilus*	*A. niger*	*C. albicans*	
6a	21 (0.95)	19 (0.95)	19 (0.90)	18 (0.85)	19 (0.90)	12.5
6b	18 (0.90)	19 (0.90)	20 (0.95)	20 (0.90)	20 (0.95)	12.5
6c	15 (0.68)	15 (0.75)	13 (0.61)	14 (0.63)	15 (0.71)	100
6d	14 (0.63)	17 (0.85)	15 (0.71)	15 (0.68)	14 (0.67)	25
6e	15 (0.68)	15 (0.75)	14 (0.66)	14 (0.63)	15 (0.71)	100
6f	19 (0.86)	17 (0.85)	17 (0.80)	20 (0.90)	18 (0.85)	25
6g	18 (0.85)	18 (0.90)	17 (0.80)	19 (0.86)	18 (0.85)	12.5
6h	12 (0.54)	14 (0.70)	12 (0.57)	15 (0.68)	16 (0.76)	100
6i	14 (0.63)	16 (0.80)	15 (0.71)	17 (0.77)	15 (0.71)	50
6j	16 (0.72)	15 (0.75)	15 (0.71)	16 (0.72)	14 (0.67)	50
Gentamycin	22	20	21	—	—	—
Nystatin	—	—	—	22	21	—
Streptomycin	—	—	—	—	—	6.25
Control (DMF)	—	—	—	—	—	—

a) Diameter of well (bore size), 6 mm; activity index = inhibition zone of the sample/inhibition zone of the standard.

were incubated at 37 °C for 24 h in case of antibacterial activity and 72 h in case of antifungal activity. The diameter of the zone of inhibition for all the test compounds was measured and the results were compared with the standard drug Gentamycin for antibacterial activity and Nystatin for antifungal activity at the same concentration (1000 μg/ml in DMF) as that of test drugs and tabulated in Table 1.

Antituberculosis Testing Method *In vitro* antituberculosis testing was carried out against the human virulent strain *M. tuberculosis* ($H37R_v$) by the method of disperse culture technique using Kirchner's medium method containing Tween-80 as described by reported method.[19] To sterile Kichner disperse medium (4.5 ml) dispersed in borosilicate test tube (150×20 mm), was added 0.5 ml of sterile normal bovine serum, inactivated by heating at 56 °C for 30 min.

The compounds **6a**—**j** under test were dissolved in DMF and added in the form of solution in such a way as to give final concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 μg per ml to the inoculums consisting of 0.1 ml of standard suspension of *M. tuberculosis* ($H37R_v$) containing 10^6 bacilli/ml. The tubes were incubated at 37 °C for 8 d and then examined for the presence or absence of the growth of the test organism. The lowest concentration that showed no visible growth was taken as an end point. The minimum inhibition concentration for all the test compounds was measured and the results were compared with the standard drug tube with streptomycin and tube with DMF used as control. The minimum inhibition concentration of all the newly synthesized compounds was tabulated in the Table 1.

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