Search for Inhibitors of Bacterial and Human Protein Kinases among Derivatives of Diazepines[1,4] Annelated with Maleimide and Indole Cycles

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Aminomethylation of 9b,10-dihydro-1*H*-indolo[1,7:4,5,6]pyrrolo[3,4:2,3][1,4]diazepino-[1,7-*a*]indole-1,3(2*H*)-diones or 1*H*-indolo[1,7:4,5,6]pyrrolo[3,4:2,3][1,4]diazepino[1,7-*a*]indole-1,3(2*H*)-diones resulted in dialkylaminomethyl derivatives. Alkylation of the nitrogen atom of maleimide moiety of polyannelated diazepines with 1,3-dibromopropane and subsequent reaction with thiourea or its *N*-alkyl derivatives gave isothiourea-carrying compounds. The compounds containing isothiourea moiety were active against individual human serine/threonine and tyrosine kinases at low micromolar concentrations. Dialkylaminomethyl derivatives of diazepines sensitized *Streptomyces lividans* with overexpressed aminoglycoside phosphotransferase type VIII (aphVIII) to kanamycin by inhibiting serine/threonine kinase(s) mediated aphVIII phosphorylation.

Introduction

Protein kinases are the key regulators of cellular signaling, thereby representing attractive targets for therapeutic intervention in a variety of diseases. These enzymes represent the largest family of signaling proteins in eukaryotic cells implicated in a plethora of aspects of cell regulation.[1,2] Protein kinases interact with diverse substrates ranging from the enzymes, including protein kinases, to transcription factors, receptors, and chromatin components. Furthermore, the bacterial eukaryotic-type serine/threonine protein kinases regulate biofilm formation and are therefore pivotal for bacterial survival.[3−5] Also, protein kinases play a key role in control of virulence and growth of *Streptococci* and *Mycobacteria*[6,7] and are involved in resistance of *Streptomyces* to aminoglycoside antibiotics.[8−10] Thus, targeting protein kinases emerges as an important component of therapeutic regimens in a variety of diseases including somatic disorders and infections.

Potent inhibitors of protein kinases, predominantly the serine/threonine type kinases, have been found among the derivatives of bis-3,4(indol-3-yl)maleimides and their congeners.[1,2] In these compounds the maleimide moiety interacts with the kinase ATP binding pocket via two hydrogen bonds.[11] We have developed the method of preparation of 3,4-bis(indol-1-yl)maleimides (**1**) that are isomeric to 3,4-bis(indol-3-yl)maleimides. 3,4-Bis(indol-1-yl)maleimides under the action of protic acids were transformed into derivatives of dihydrodiazepines[1,4], 9b,10-dihydro-1*H*-indolo[1,7:4,5,6]pyrrolo[3,4:2,3][1,4]diazepino[1,7-*a*]indole-1,3(2*H*)-diones (**2**). Dehydrogenation of **2** leads to 1*H*-indolo[1,7:4,5,6]pyrrolo[3,4:2,3][1,4]diazepino[1,7-*a*]indole-1,3(2*H*)-diones (**3**) (Scheme 1).[12] The conformations of annelated diazepines **2** and **3** are shell-like and therefore differ substantially from the conformation of planar maleimidoindolocarbazoles formed from 3,4-bis(indol-3-yl)maleimides.[13] This suggests that the spectrum of protein kinases inhibited by annelated diazepines might vary from that of annelated maleimidoindolocarbazoles.

In this study we developed the methods of preparation of diazepine derivatives **2** and **3**, aiming at identification of serine/threonine and tyrosine protein kinase inhibitors within this chemical class. The compounds with the dialkylaminomethyl group in position 3 of the indole moiety of **2** or **3**, as well as the compounds containing a substituent at the nitrogen atom of the maleimide cycle, were synthesized. We tested our novel compounds for the ability to inhibit human protein kinases in vitro and protein kinase signaling in *Streptomyces*. Our results provide evidence that individual derivatives of diazepines[1,4] annelated with maleimide and indole cycles are differentially potent against human and bacterial protein kinases. Thus, some hit compounds might be perspective anti-infective agents, while the others are promising as candidate drugs for somatic diseases.

Chemistry

Introduction of Substituents at Position 3 of the Indole Moiety. Aminomethylation (Mannich reaction) of compounds **2a** or **2b** under the action of dialkylamines and paraformaldehyde in acetic acid led to **4a**, **4b**, or **5b** in approximately 50% yields. Aminomethylation of diazepine **3b** using morpholine produced bis-morpholinomethyl derivative **6** (50% yield) (Scheme 2).

Introduction of Substituents at the Nitrogen Atom of the Maleimide Cycle. The interaction of diazepine[1,4] **2a** with 1,3-dibromopropane in boiling dioxane in the presence of K_2CO_3 yielded compound **7**, which was separated by chromatography in 50% yield. The interaction of **7** with $HNEt_2$ or *N*-methylpiperazine in DMF led to **9a** or **9b** in 70% yields. Condensation of **7** with thiourea or N-substituted thioureas in boiling ethanol−dioxane yielded compounds **10a**−**f** (Scheme 3). 3-Bromopropyl derivative **8** was obtained from diazepine **3a** used for preparation of **11a**,**b** that contain the isothiourea moiety

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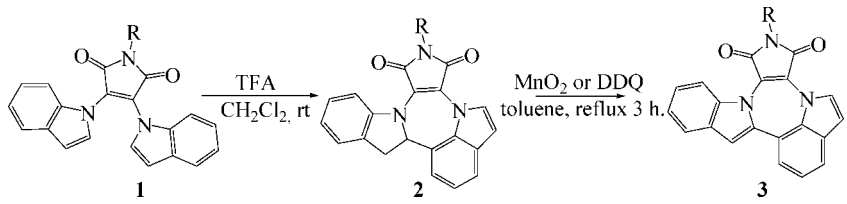
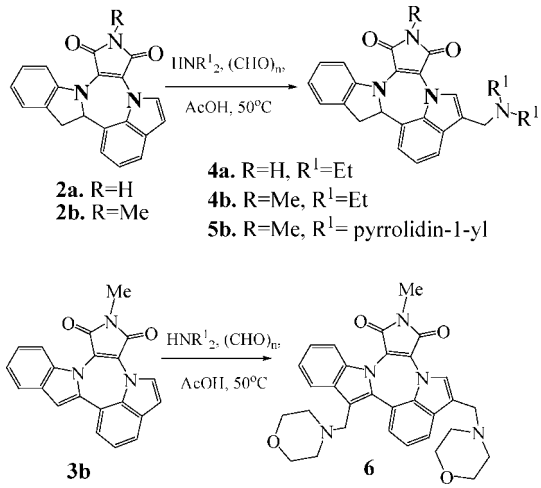
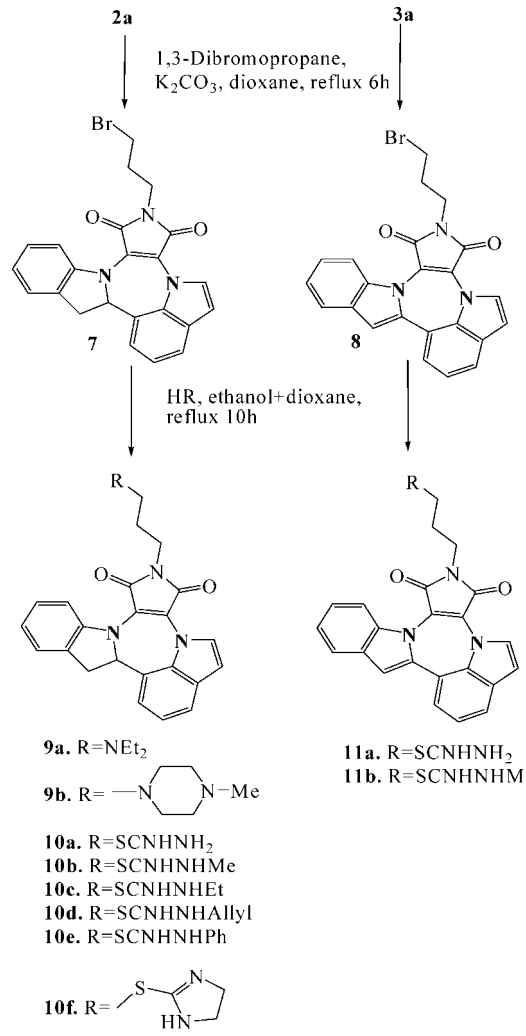
Scheme 1. Bis(3,4-indol-1-yl)maleimides (**1**) and Their Transformation into Annelated Maleimidoindolodiazepines 9b,10-dihydro-1*H*-indolo[1,7:4,5,6]pyrrolo[3,4:2,3][1,4]diazepino[1,7-*a*]indole-1,3(2*H*)-diones (**2**) and 1*H*-indolo[1,7:4,5,6]pyrrolo[3,4:2,3]-[1,4]diazepino[1,7-*a*]indole-1,3(2*H*)-diones (**3**)



Scheme 2. Aminomethylation of Compounds **2** and **3**



Scheme 3. Synthesis of N-Substituted Polyannelated Maleimidoindolodiazepines[1,4]



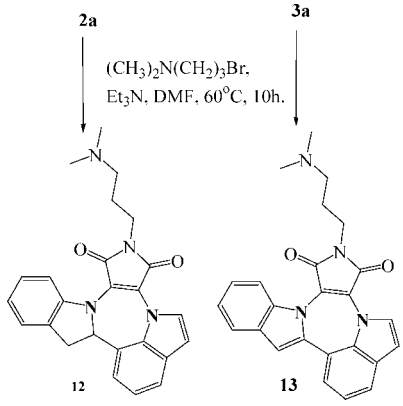
(Scheme 3). Interaction of **3a** with 1-(*N*,*N*-dimethylamino)-3-chloropropane in boiling dioxane in the presence of K_2CO_3 gave **13** in 70% yield. Similarly, compound **12** was obtained from compound **2a** (Scheme 4). Properties of the new compounds are available in Supporting Information.

Biological Evaluation

Inhibitory Activities of Novel Compounds against Human Protein Kinases. The compounds were tested against a panel of 25 human protein kinases (AKT1, ARK5, Aurora-A, Aurora-B, B-RAF-V600E, CDK2/CycA, CDK4/CycD1, CK2-α1, COT, INS-R, MET, PDGFR-β, PLK1, SAK, SRC, TIE2, AXL, EGF-R, EPHB4, ERBB2, FAK, IGF1-R, VEGF-R2, VEGF-R3, and PKC-α). The compounds containing the dialkylaminomethyl substituent at the position 3 of indole ring(s) (**4**−**6**) or dialkylaminopropyl substituent at the position 1 of maleimide ring (**9**, **12**, and **13**) were inactive against tested enzymes at micromolar concentrations. In constrast, the compounds containing a propyl carbamimidothioate substituent at the maleimide nitrogen (**10** and **11**) attenuated the activity of individual kinases. The inhibitory activities of these compounds are presented in Table 1. It is worth noting that propyl carbamimidothioate derivatives of diazepine[1,4] **2** (**10a**,**c**,**d**,**f**) were more specific toward cyclin dependent kinases (CDKs) than the isothiourea-containing derivatives of **3** (**11a**,**b**). The latter compounds were less selective toward the kinases in the panel. Compounds **10** and **11** represent the maleimide derivatives substituted at the maleimide nitrogen atom. It has been reported that substitution of maleimide nitrogen of indolylmaleimide derivatives leads to the loss of the activity against protein kinases.[14] To investigate the binding mode of N-substituted bis-indolylmaleimides in the active site of protein kinases, molecular docking was applied.

Molecular Docking of 10a and 11a to CDK2. Binding of **10a** and **11a** with CDK2, a well-studied kinase representative, was modeled. Results of the docking studies showed that the preferential mode of binding of **10a** and **11a** to CDK2 was characterized by single hydrogen bond formed by maleimide carbonyl oxygen and main chain amide of hinge residue Leu83. However, this hydrogen bond was not observed for all CDK2 models. When it was present (7 cases out of 11), this bond was relatively long (∼2.5 Å), suggesting that binding of N-substituted bis-indolylmaleimides was not as specific compared to other kinase inhibitors that form at least two hydrogen bonds with the hinge region.[11] Nevertheless, although docking to particular CDK2 models revealed some alternative binding modes of **10a** and **11a**, the described binding via single conservative hydrogen bond prevailed. Moreover, this mode of interaction was characterized by higher binding energy. The

Scheme 4. Synthesis of Compounds **12** and **13**

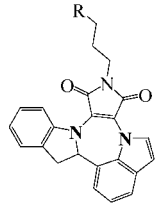
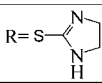
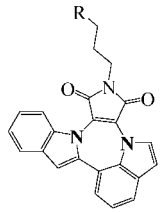


proposed mode of binding of **10a** and **11a** with CDK2 is depicted in Figure 1.

New Bacterial Cell-Based Test System for Screening of Protein Kinase Inhibitors. Principle of the Test System. It has been demonstrated[8] that the resistance to aminoglycoside antibiotics (e.g., kanamycin) of a *Streptomyces lividans* strain with exogenously expressed aminoglycoside phosphotransferase type VIII (aphVIII), the enzyme that inactivates kanamycin, is mediated by a serine/threonine kinase(s) that up-regulates aphVIII. This protein contains Ser[146], the site of phosphorylation for serine/threonine protein kinases of *Streptomyces coelicolor* or *Streptomyces lividans*.[8,9] Phosphorylation of aphVIII at this site results in an increased resistance of *Streptomyces lividans* aphVIII+ to kanamycin. Thirty-four serine/threonine protein kinases have been identified in *Streptomyces coelicolor* A(3)2 strain closely related to *Streptomyces lividans* 66.[15] At least one Ca^{2+}-dependent serine/threonine protein kinase in *Streptomyces coelicolor*, pk25 (as well as its homologue in *Streptomyces lividans)* can phosphorylate aphVIII.[8] Thus, sensitization of bacteria to kanamycin, the phenomenon dependent on aphVIII phosphorylation, can be used for testing the ability of low molecular weight compounds to attenuate the activity of protein kinase(s) in a *Streptomyces*-based test system. If the protein kinase(s) is inhibited, the aphVIII is not phosphorylated and the zone of bacterial lysis is increased compared with control (no inhibitor). Together, these data strongly suggest that the inhibitors of serine/threonine protein kinases can sensitize *Streptomyces lividans* aphVIII+ to kanamycin via attenuation of serine/threonine protein kinase(s) mediated aphVIII phosphorylation. Our derivatives can sensitize *Streptomyces* to kanamycin (see below), whereas the effect of sensitization was not observed in a *E. coli* aphVIII+ strain,[10] the bacteria that possess no eukaryotic-type serine/threonine protein kinases.[16]

Validation of the Bacterial Test System. We examined the ability of partially purified protein kinases of *Streptomyces coelicolor* to phosphorylate aphVIII. A radiometric protein kinase assay was used for determination of the kinase activity of actinobacterial protein kinases in vitro. Protein kinases were incubated in standard conditions in the presence of [γ-^{32}P]ATP and aphVIII with or without known modulators and inhibitors of protein kinase activity and incorporation of γ-phosphoryl residue into aphVIII was determined. The results (Table 1-SI

Table 1. Inhibition of Human Protein Kinases by Isothiourea Containing Compounds[a]

Compound	Structure	R	Protein kinases inhibited (IC_{50}, μM)*
10a		R=SCNHNH$_2$	CDK2/Cyc2 (4.3)
10b		R=SCNHNHMe	B-RAF-V600E (8), CDK2/CycA (5.3), CDK4/CycD1 (5.7), IN S-R (5.1), MET (6.7)
10c		R=SCNHNHEt	CDK2/CycA (6.8)
10d		R=SCNHNHAllyl	CDK4/CycD1 (6.9)
10f			CDK2/CycA (7.7)
10e		R=SCNHNHPh	CDK2/CycA (5.1), CDK4/CycD1 (7.9), SRC (2.5), TIE2 (8.9), EGF-R (9.5), IGF1-R (2.3), VEGF-R2 (4.3), VEGF-R3 (9.6)
11a		R=SCNHNH$_2$	AKT1 (9.0), B-RAF-V600E (1.2), CDK2/CycA (3.6), CDK4/CycD1 (2.7), INS-R (2.6), MET (3.3), PDGFR-β (4.2), PLK1 (9.9), SRC (3.8), AXL (4.4), EGFR (4.6), EPHB4 (2.3), ERBB2 (7.7), FAK (4.4), IGF1-R (4.0), VEGF-R2 (4.1), VEGF-R3 (4.7)
11b		R=SCNHNHMe	ARK5 (9.7), B-RAF-V600E (8.4), CDK2/CycA (6.3), CDK4/CycD1 (6.2), INS-R (4.6), MET (7.4), PDGFR-beta (6.7), ErbB2 (10) (9.9), EPHB4 (3.9)

[a] (*) IC_{50}, the concentration of the inhibitor that down-regulated the kinase activity by 50% (mean of three independent measurements). The kinase activity in the absence of the inhibitor was considered as 100%.

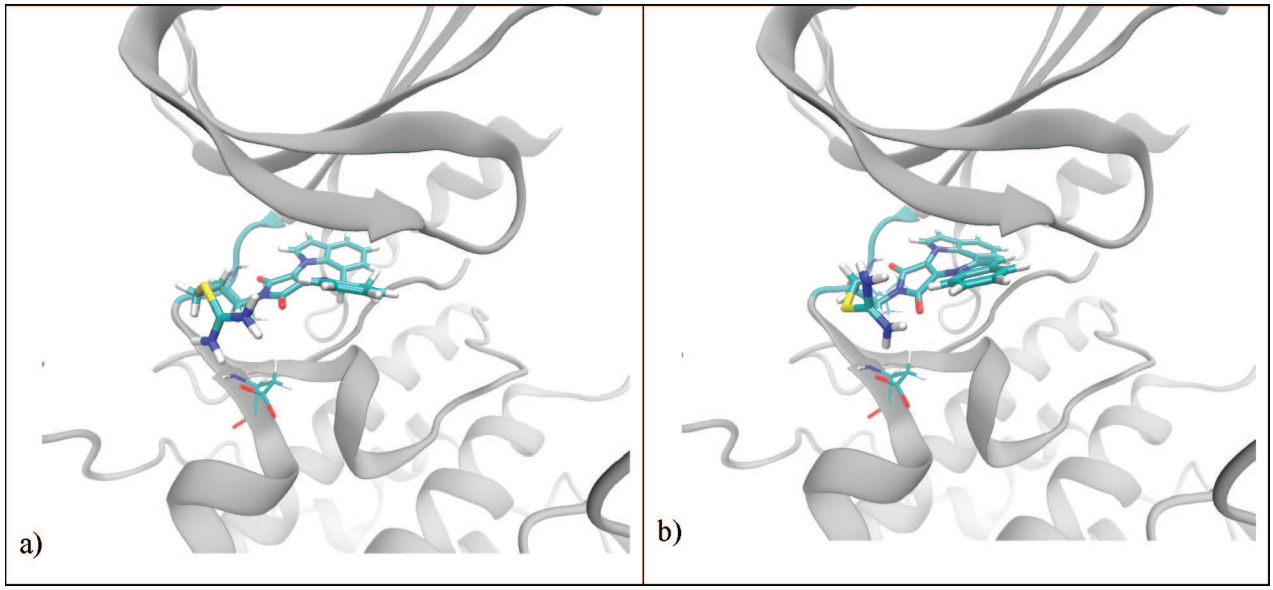


Figure 1. Binding of **10a** (a) and **11a** (b) to the ATP-binding pocket of CDK2 predicted by molecular docking. The model was prepared from PDB structure 1r78.

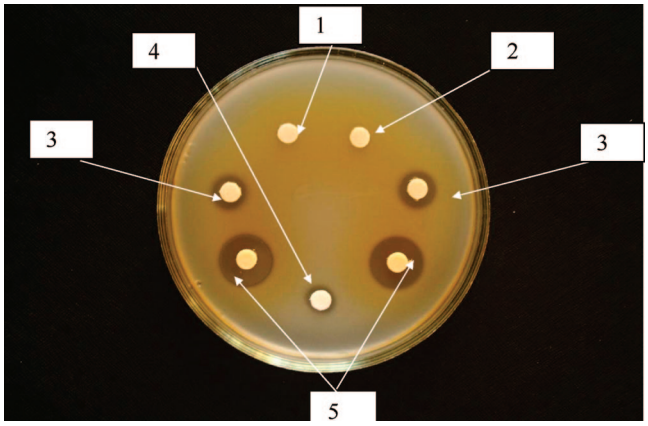


Figure 2. Growth inhibition of *Streptomyces lividans* aphVIII+ strain: (1) kanamycin, 5 nM/disk; (2) Bis-I, 300 nM/disk; (3) Bis-I + kanamycin, halo diameter = 10 mm; (4) **4b**, 100 nM/disk; (5) **4b** + kanamycin, halo diameter = 14 mm.

in Supporting Information) indicated that at least three aphVIII-phosphorylating activities, Ca^{2+}-independent, Ca^{2+}/calmodulin-dependent, and Ca^{2+}/phospholipid-dependent, were detectable in the *Streptomyces coelicolor* cell lysates. The selective inhibitor of Ca^{2+}/calmodulin-dependent protein kinases, KN-62,[a] blocked aphVIII phosphorylation, whereas its inactive analogue, KN-92, had no effect. Bis-I, the inhibitor of Ca^{2+}/phospholipid-dependent serine/threonine protein kinases, attenuated Ca^{2+}/phospholipid-dependent aphVIII phosphorylation by approximately 50%, whereas Bis-V, a compound with little kinase inhibitory potency, evoked only marginal effect on aphVIII phosphorylation. Importantly, compound **4b** decreased aphVIII phosphorylation down to the basal level (Table 3-SI in Supporting Information). Next, we tested the ability of Bis-I, Bis-V, **4a**, **4b**, and **9a** to influence aphVIII induced kanamycin phosphorylation. As shown in Table 3-SI (Supporting Information), none of these compounds had a discernible effect on kanamycin phosphorylation, indicating that bis-indolylmaleimides and our novel compounds do not target kanamycin phosphorylation by aphVIII in vitro. The present data validated our bacterial test system as an appropriate tool for phenotypic screening of protein kinase inhibitors.

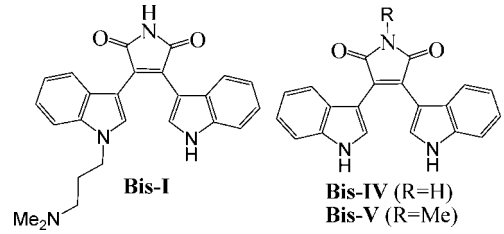
Use of the Bacterial Test System for Screening of Protein Kinase Inhibitors. We next used our bacterial test system for screening novel compounds for their ability to inhibit

Table 2. Inhibition of Growth of *Streptomyces lividans* aphVIII+ Strain by Polyannelated Diazepine Derivatives

compd	subtoxic concentration, nM/disk[a]	halo diameter (mm), kanamycin 5 μg/disk[b] + inhibitor
4a	25	13.0 ± 0.5
4b	100	14.0 ± 0.5
5b	200	13.0 ± 0.5
9a	20	12.5 ± 0.5
13	400	12.5 ± 0.5
12	100	11.5 ± 0.5
10a	2000	11.5 ± 0.5
10c	40	10.5 ± 0.5
10b	100	10.5 ± 0.5
10d	100	10.0 ± 0.5
11a	2700	10.0 ± 0.5
10f	40	9.0 ± 0.5
11b	1000	9.0 ± 0.5
10e	1500	9.0 ± 0.5
Bis-I	300	10 ± 0.5
Bis-V	3	7.5 ± 0.5

[a] Minimal toxic concentration was 2-fold higher than the concentration used in the experiments. [b] Kanamycin alone did not inhibit growth of the test culture (halo diameter = 0).

Chart 1. Bis(indol-3-yl)maleimides Bis-IV, Bis-V, and Bis-I



[a] Abbreviations: Bis-I: [1-(3-dimethylaminopropyl)-1*H*-indol-3-yl]-4-(1*H*-indol-3-yl)pyrrole-2,5-dione; Bis-V: 1-methyl-3,4-bis(1*H*-indol-3-yl)pyrrole-2,5-dione; KN-62, [(1-[*N*,*O*-bis(5-isoquinolinesulphonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine); KN-92, 2-[*N*-(4′-methoxybenzenesulfonyl]amino-*N*-(4′-chlorophenyl)-2-propenyl-*N*-methylbenzylamine phosphate; EGTA, ethylene glycol bis(β-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

protein kinase signaling by sensitizing *Streptomyces lividans* aphVIII+ strain to kanamycin. Figure 2 shows that kanamycin alone had virtually no growth inhibitory effect, nor did we observe bacterial lysis by **4b** or Bis-I alone. In constrast, **4b** in combination with kanamycin produced a zone of bacterial lysis 14 mm in diameter. A smaller size of lytic halo was observed around the paper disk imbibed with both kanamycin and the reference protein kinase inhibitor Bis-I (Figure 2). This representative experiment demonstrated that our bacterial test system can be used to study the kinase inhibitory activity of low molecular weight compounds. Each compound was tested at a subtoxic dose, i.e., at the concentration ~50% of minimal growth inhibitory dose. Therefore the concentrations of individual compounds (Table 2) were equitoxic (see also Supporting Information, Table 4—SI, for detailed dose response). As shown in Table 2, the compounds can be divided into two groups depending on their potency. The first group included **4a**,**b**, **5b**, **9a**, and **13**, with the growth inhibition zone (halo) 12.5−14 mm in diameter. It is interesting to note also that the compounds **4b** and **5b**, bearing the methyl group at the maleimide nitrogen, were as active as unsubstituted derivative **4a**. Other compounds including Bis-I demonstrated a lower activity (growth inhibition zones smaller than 11.5 mm). Importantly, the compounds **4a**,**b**, **5b**, **9a**, and **13**, which were the most potent in the bacterial test system, were less active against human protein kinases (Table 1). These latter compounds, being the most active modulators of serine/threonine protein kinase/aphVIII mediated bacterial response to kanamycin, are of special interest because they might be candidates for future nontoxic inhibitors of pathogenicity and virulence in clinical bacterial strains.[6,7] In contrast, the isothiourea containing compounds **10a**−**f**, **11a**, and **11b** that inhibited human protein kinases (Table 1) were less potent against bacterial protein kinase signaling in our test system (Table 2). Table 3-SI (Supporting Information) shows the concentrations of novel compounds used in combination with 5 μg/disk of kanamycin. From this table it is clear that our compounds at the concentrations presented in Table 2 did not inhibit growth of *Streptomyces lividans* aphVIII+, thereby ruling out a possibility that novel compounds alone (i.e., in the absence of kanamycin) could influence the bacterial viability. Finally, none of the tested novel compounds influenced proliferation or viability of human epithelial (colon, breast) and lymphoid cells at 50 μM for 72 h of continuous cell exposure (not shown).

Concluding Remarks

In this study we report the synthesis of novel derivatives of diazepines[1,4] annelated with maleimide and indole cycles as tentative inhibitors of bacterial and human protein kinases. We developed a new *Streptomyces*-based test system for testing the kinase inhibitory potency of novel compounds. Together with the conventional in vitro kinase assay using recombinant kinases and inhibitors, the bacterial test system allowed us to specify the compounds preferentially potent for microbial or human protein kinases. Such initial screening may be useful as an informative and inexpensive tool for selection of active compounds among large scale chemical libraries.

Experimental Section

Chemistry. 6-[(Diethylamino)methyl]-9b,10-dihydro-1*H*-indolo[1′,7′:4,5,6]pyrrolo[3′,4′:2,3][1,4]diazepino[1,7-*a*]indole-1,3(2*H*)-dione (4a). To a stirred solution or suspension of diazepine **2a** (800 mg, 2.4 mmol) in AcOH (50 mL) were added paraformaldehide (500 mg) and $HNEt_2$ (0.73 g, 10 mmol). The mixture was stirred at 50 °C for 20 h, concentrated in vacuo, diluted with EtOAc (100 mL), washed with saturated aqueous $NaHCO_3$ to neutral pH, saturated aqueous NaCl (50 mL), dried, and evaporated. The residue after solvent evaporation was chromatographed ($CHCl_3$−MeOH, 10:1) to give **4a** as a violet amorphous compound (540 mg, 1.3 mmol, 55%): δ_H (DMSO-d_6) 1.00−1.04 (6H, m), 2.54−2.56 (4H, m), 3.67−3.83 (4H, m), 5.21−5.25 (1H, m), 6.79 (1H, t, J = 7.51), 6.84 (1H, d, J = 7.87), 7.01 (1H, t, J = 8.87), 7.18 (1H, t, J = 7.51), 7.23 (1H, d, J = 7.33), 7.29 (1H, d, J = 7.69), 7.74 (1H, d, J = 7.68), 8.39 (1H, s); ESI-HRMS calculated for ($C_{25}H_{24}N_4O_2$ + H^+) 413.1972, found 413.1976; purity 98.7% (HPLC).

6-[(Diethylamino)methyl]-2-methyl-9b,10-dihydro-1*H*-indolo[1′,7′:4,5,6]pyrrolo[3′,4′:2,3][1,4]diazepino[1,7-*a*]indole-1,3(2*H*)-dione (4b). Compound **4b** was obtained from **2b** (800 mg, 2.3 mmol) as described for **4a**. The residue after solvent evaporation was chromatographed (hexane−EtOAc−Et_3N, 3:1:0.1) to give **4b** as a violet solid (640 mg, 1.5 mmol, 65%); mp 86−88 °C (*i*-PrOH); δ_H ($CDCl_3$) 1.05−1.09 (6H, m), 2.52−2.58 (4H, m), 3.10 (3H, s), 3.69 (2H, s), 3.79−3.81 (2H, m), 5.10−5.13 (1H, m), 6.81−6.85 (2H, m), 7.07 (1H, t, J = 8.59), 7.19−7.22 (3H, m), 7.77−7.79 (1H, m), 8.43 (1H, s); ESI-HRMS calculated for ($C_{26}H_{26}N_4O_2$ + H^+) 427.2129, found 427.2123; purity 99.1% (HPLC).

2-Methyl-6-(1-pyrrolidinylmethyl)-9b,10-dihydro-1*H*-indolo[1′,7′:4,5,6]pyrrolo[3′,4′:2,3][1,4]diazepino[1,7-*a*]indole-1,3(2*H*)-dione (5b). Compound **5b** was obtained from **2b** (800 mg, 2.3 mmol) as described for **4a**. The residue after solvent evaporaition was chromatographed (hexane−EtOAc−Et_3N, 3:1:0.1) to give **5b** as a violet solid (640 mg, 1.5 mmol, 65%): mp 69−71 °C (*i*-PrOH); δ_H ($CDCl_3$) 1.74−1.77 (4H, m), 2.54−2.57 (4H, m), 3.09 (3H, s), 3.77−3.82 (2H, m), 5.07−5.10 (2H, m), 6.81−6.84 (2H, m), 7.07 (1H, t, J = 6.77), 7.21−7.22 (2H, m), 7.71−7.73 (2H, m) 8.45 (1H, s); ESI-HRMS calculated for ($C_{26}H_{24}N_4O_2$ + H^+) 425.1972, found 425.1978; purity 98.4% (HPLC).

3-(1,3-Dioxo-1,3,9b,10-tetrahydro-2*H*-indolo[1′,7′:4,5,6]pyrrolo[3′,4′:2,3][1,4] diazepino[1,7-*a*]indol-2-yl)propyl Imidothiocarbamate (10a). The stirred solution or suspension of diazepine **7** (500 mg, 1.1 mmol) in EtOH (20 mL) was boiled for 7 h with thiourea (250 mg, 3.3 mmol), cooled, and concentrated in vacuo, diluted with EtOAc (50 mL), washed with saturated aqueous NaCl (50 mL), dried, and evaporated. The residue after solvent evaporaition was chromatographed ($CHCl_3$−MeOH, 10:1) to give **10a** as a violet amorphous compound (240 mg, 0.55 mmol, 50%): δ_H (DMSO-d_6) 1.88−1.92 (2H, m), 2.89−2.93 (2H, m), 3.58−3.62 (2H, m), 3.72−3.79 (2H, m), 5.27−5.31 (1H, m), 6.82 (1H, t, J = 7.32), 6.85 (1H, d, J = 3.48), 6.89 (1H, d, J = 7.87), 7.04 (1H, t, J = 7.29), 7.20 (1H, t, J = 7.65), 7.26 (1H, d, J = 7.32), 7.32 (1H, d, J = 7.65), 7.65 (1H, d, J = 7.83), 8.45 (1H, d, J = 3.62), 9.62 (3H, s); ESI-HRMS calculated for ($C_{24}H_{21}N_5O_2S$ + H^+) 444.1489, found 444.1482; purity 95.2% (HPLC).

3-(1,3-Dioxo-1,3,9b,10-tetrahydro-2*H*-indolo[1′,7′:4,5,6]pyrrolo[3′,4′:2,3][1,4] diazepino[1,7-*a*]indol-2-yl)propyl *N*-Methylimidothiocarbamate (10b). Compound **10b** was obtained from **7** (500 mg, 1.1mmol) as described for **10a**. The residue was chromatographed ($CHCl_3$ − MeOH, 10:1), to give **10b** as a violet amorphous compound (240 mg, 0.55 mmol, 50%); δ_H (DMSO-d_6) 1.93−1.97 (2H, m), 2.91 (3H, s), 3.28−3.32 (2H, m), 3.62−3.65 (2H, m), 3.75−3.91 (2H, m), 5.33−5.36 (1H, m), 6.82 (1H, t, J = 7.43), 6.87 (1H, d, J = 3.63), 6.90 (1H, d, J = 8.06), 7.03 (1H, t, J = 7.28), 7.21 (1H, t, J = 7.68), 7.27 (1H, d, J = 7.23), 7.35 (1H, d, J = 7.69), 7.67 (1H, d, J = 7.83), 8.44 (1H, d, J = 3.48), 9.40 (2H, s); ESI-HRMS calculated for ($C_{25}H_{23}N_5O_2S$+ H^+) 458.1645, found 458.1649; purity 96.5% (HPLC).

3-(1,3-Dioxo-1,3-dihydro-2*H*-indolo[1′,7′:4,5,6]pyrrolo[3′,4′:2,3][1,4]diazepino[1,7-*a*]indol-2-yl)propyl Imidothiocarbamate (11a). Compound **11a** was obtained from **8** (500 mg, 1.1mmol) as described for **10a**. The residue was chromatographed ($CHCl_3$−MeOH, 10:1) to give **11a** as a red amorphous compound (240 mg, 0.55 mmol, 50%): δ_H (DMSO-d_6) 1.90−1.93 (2H, m), 3.19−3.22 (2H, m), 3.55−3.61 (2H, m), 6.78 (1H, d, J = 3.66), 7.08 (1H, t, J = 7.14), 7.11−7.18 (3H, m), 7.45−7.49 (3H, m), 7.70 (1H, d, J = 7.73), 8.11 (1H, d, J = 3.62), 9.09 (3H, s). ESI-HRMS calculated for ($C_{24}H_{19}N_5O_2S$ + H^+) 442.1332, found 442.1337; purity 98.9% (HPLC).

3-(1,3-Dioxo-1,3-dihydro-2*H*-indolo[1′,7′:4,5,6]pyrrolo[3′,4′:2,3][1,4]diazepino[1,7-*a*]indol-2-yl)propyl *N*-Methylimidothiocarbamate (11b). Compound **11b** was obtained from **8** (500 mg, 1.1mmol) as described for **10a**. The residue was chromatographed ($CHCl_3$−MeOH, 10:1) to give **11b** as a red amorphous compound (240 mg, 0.55 mmol, 50%): δ_H (DMSO-d_6) 1.89−1.91 (2H, m), 2.89 (3H, s), 3.17−3.20 (2H, m), 3.54−3.61 (2H, m), 6.77 (1H, d, $J = 3.68$), 7.06 (1H, t, $J = 7.13$), 7.10−7.17 (3H, m), 7.43−7.48 (3H, m), 7.69 (1H, d, $J = 7.70$), 8.10 (1H, d, $J = 3.58$), 9.07 (2H, s); ESI-HRMS calculated for ($C_{25}H_{21}N_5O_2S+H^+$) 456.1489, found 456.1483; purity 99.0% (HPLC).

Molecular Modeling. Binding of **10a** and **11a** with CDK2, a representative protein kinase, was modeled in the following way. Three dimensional structures of **10a** and **11a** were modeled at the Hartree−Fock level of theory (semiempiric AM1 method) using the Gaussian 98 program package.[17] The full atomic models of CDK2 structure were prepared from the available Protein Data Bank (PDB) structures using Lead Finder software.[18] In total, 11 models were prepared starting from PDB files 1gz8, 1h00, 1ke9, 1oit, 1r78, 2aoc, 2b52, 2b55, 2r3h, 2r3r, 2vtt to imitate the ATP binding site flexibility. The set of different CDK2 structures was selected from PDB using the criteria of good structure resolution (<2 Å) and chemical diversity of bound ATP-competitive inhibitors. Protein−ligand docking was performed with Lead Finder software.

Biological Methods. The protocol of in vitro kinase assay is given in Supporting Information. A strain of *Streptomyces lividans* harboring pSU23 plasmid carrying the *aphVIII* gene (*Streptomyces lividans* aphVIII+ strain) was used as a test culture to analyze the inhibitors of bacterial serine−treonine protein kinases. The gene product, aminoglycoside phosphotransferase aphVIII, phosphorylates and inactivates kanamycin, thereby rendering bacteria resistant to this antibiotic. This activity of aphVIII is dependent on phosphorylation by a serine/threonine protein kinase(s). The kinase inhibitory activity of new compounds was investigated by the paper disk method. Paper disks (7 mm in diameter) containing kanamycin (5 μg/disk) and various amounts of tested compounds were applied on the plates with logarithmically growing *Streptomyces lividans* aphVIII+ and incubated at 28 °C for 20 h. The halo diameters formed after exposure of bacteria with the combination of kanamycin and potential inhibitors were compared with the respective sizes for kanamycin alone or the combinations of kanamycin and known inhibitor of serine/threonine protein kinases, Bis-I.[19] As a negative control, Bis-V was used.[20] The structures of Bis-I and Bis-V are shown in Chart 1. To rule out the cytotoxicity of novel compound as a factor that might increase the diameter of the zone of lysis, we used subtoxic concentrations of tested compounds. The subtoxic concentrations were approximately 2 times lower than the minimal toxic concentrations, i.e., minimal doses that inhibited growth of test bacteria. At subtoxic concentrations no inhibition of bacterial growth was observed. The detailed methods are given in Supporting Information.

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Supporting Information Available: General experimental information and details of the synthesis of compounds **6**−**8**, **9a**,**b**, **10c**−**f**, **12**, and **13**; MS, HRMS, HPLC data of the novel compounds; details of the in vitro kinase assays; data on bacterial sensitization to kanamycin depending on the concentration of protein kinase inhibitors. This material is available free of charge via the Internet at http://pubs.acs.org.

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