HHS Public Access

Author manuscript

Bioorg Med Chem. Author manuscript; available in PMC 2018 August 24.

Published in final edited form as:

Bioorg Med Chem. 2014 March 01; 22(5): 1708–1725. doi:10.1016/j.bmc.2014.01.020.

Discovery and optimization of a new class of pyruvate kinase inhibitors as potential therapeutics for the treatment of methicillin-resistant *Staphylococcus aureus* infections

Nag S. Kumar[a], **Edie M. Dullaghan**[b], **B. Brett Finlay**[c,d], **Huansheng Gong**[d], **Neil E. Reiner**[d,e], **J. Jon Paul Selvam**[a], **Lisa M. Thorson**[d], **Sara Campbell**[b], **Nicholas Vitko**[f], **Anthony R. Richardson**[f], **Roya Zoraghi**[d], and **Robert N. Young**[a,*]

[a]Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada

[b]Centre for Drug Research and Development (CDRD), Vancouver, BC, Canada

[c]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada

[d]Department of Medicine, Division of Infectious Diseases, University of British Columbia, Vancouver, BC, Canada

[e]Department of Microbiology and Immunology, Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada

[f]Department of Microbiology and Immunology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Abstract

A novel series of bis-indoles derived from naturally occurring marine alkaloid **4** were synthesized and evaluated as inhibitors of methicillin-resistant *Staphylococcus aureus* (MRSA) pyruvate kinase (PK). PK is not only critical for bacterial survival which would make it a target for development of novel antibiotics, but it is reported to be one of the most highly connected ‘hub proteins’ in MRSA, and thus should be very sensitive to mutations and making it difficult for the bacteria to develop resistance. From the co-crystal structure of *cis*-3–4-dihydrohamacanthin B (**4**) bound to *S. aureus* PK we were able to identify the pharmacophore needed for activity. Consequently, we prepared simple direct linked bis-indoles such as **10b** that have similar anti-MRSA activity as compound **4**. Structure–activity relationship (SAR) studies were carried out on **10b** and led us to discover more potent compounds such as **10c, 10d, 10k** and **10m** with enzyme inhibiting activities in the low nanomolar range that effectively inhibited the bacteria growth in culture with minimum inhibitory concentrations (MIC) for MRSA as low as 0.5 μg/ml. Some potent PK inhibitors, such as **10b**, exhibited attenuated antibacterial activity and were found to be substrates for an efflux mechanism in *S. aureus*. Studies comparing a wild type *S. aureus* with a construct (*S. aureus* LAC Δ*pyk*::Erm^R) that lacks PK activity confirmed that bactericidal activity of **10d** was PK-dependant.

*Corresponding author. Tel.: +1 (778) 782 3351; fax: +1 (778) 782 3765. robert_young@sfu.ca (R.N. Young).

Keywords

Antibacterial; MRSA; Pyruvate kinase; Bis-indole

1. Introduction

Multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), have developed numerous resistance mechanisms in response to antibiotic pressure.[1] There is an increasing incidence of MRSA infections in hospitals worldwide and they have begun to penetrate into the general community. The vast majority of current antibiotics in use are directed to critical proteins unique to the bacteria and without human homologs to avoid mechanism based toxicity. This has severely limited the available targets for drug design.

Recently, pyruvate kinase (PK) was identified as a highly interconnected essential hub protein in MRSA, with structural features distinct from the human homologs, as a novel drug target.[2–4] This was based on the supposition that hub proteins are not only critical for bacterial survival but should be very sensitive to mutations[5] and targeting them should reduce the potential for development of resistance strains and species. In silico library screening, initially directed to putative binding sites unique to MRSA PK, combined with enzyme assays identified several active MRSA PK inhibitors including compound **1**[6] (Fig. 1). Compound **1** was very selective for the bacterial enzyme compared to four human PK isoforms (M1, M2, R and L) and it did not inhibit growth of HeLa cells indicating a lack of overt toxicity to mammalian cells. Structure–activity relationship (SAR) studies were initiated which led to the identification of more potent enzyme inhibitors (such as **2**) and which showed effective inhibition of a wide panel of gram positive bacterial growth, with potencies comparable to standard antibiotics such as vancomycin.[3,7] In addition, the MIC was not significantly increased even after 25 bacterial passages in culture with compound **2** at the highest sub-lethal concentration,[4] which confirmed that MRSA PK is an essential target less prone to developing resistance. X-ray crystal structures for **1** and **3** bound to MRSA282 PK were obtained which revealed that both compounds bind to a flat lipophilic pocket at the minor interfaces in the homo-tetrameric enzyme structure. This pocket was found to be modified and not accessible in the human PK enzymes.

More recently, Zoraghi et al.[8] screened the inhibitory potential of a natural marine product library of 968 crude benthic invertebrate extracts and identified *cis*-3–4-dihydrohamacanthin B (**4**) as a potent inhibitor of MRSA PK with an IC_{50} of 16 nM. Compound **4** also exhibited anti-bacterial activity with MIC of 12.5 μg/ml (tested against *S. aureus* stains RN4229 and MRSA252). They were able to derive an X-ray crystal structure of **4** bound to MRSA PK and found that it binds to the same site as the hydrazone compounds **1** and **3**.

From examination of the crystal structure of *cis*-3–4-dihydro-hamacanthin B (**4**) bound to *S. aureus* PK, it is apparent that the two indole moieties lie in a linear relationship to each other and are essentially in the same plane. The compound is anchored by symmetric hydrogen bonds between Ser362 and Ser365 from chains A and B, respectively, and the indole nitrogens (Fig. 2a). The indole phenyl rings have prominent hydrophobic interactions with

Ile361 and His365. The two bromine atoms are oriented towards the interior of the binding site in the deep hydrophobic pocket formed by Thr353, Ser354, Ala358, and Leu370. The symmetrical nature of the binding pocket was mirrored by the pseudo-symmetrical properties of the ligand. Hence it appears that both the indoles in the scaffold are critical for binding and the structure suggested that by linking the two indole at the C-2 position and removing the lactam ring of **4**, one might derive compounds such as compound **10b** (Fig. 2b) having all the necessary elements to bind tightly to MRSA PK.

In this paper, we present a detailed account of SAR for enzyme inhibitory and optimization of antibacterial activity for such an extensive series of bis-indoles.

2. Results

2.1. Chemistry

The syntheses of all target compounds were carried out as described in Schemes 1–6. The indole NH was first protected with a phenylsulfonyl group to give intermediate **6** which was subsequently iodinated at the 2-position to give 2-iodoindole **7** by treating **6** with LDA followed by the addition of diiodoethane (Scheme 1). We did attempt to couple **7** with the boronic acid **9** under standard Suzuki–Miyura conditions but no desired product was isolated. Fortunately, the coupling reaction of boronic acid **9** with the unprotected indole **8**, (obtained by hydrolysis of compound **7**), proceeded smoothly to give the desired adduct. Finally, removing the Boc protecting group with TFA gave the desired compound **10**. In order to prepare the alkylated bis-indole **12, 8** was first alkylated with alkyl halide to give intermediate **11**, which was subsequently coupled with boronic acid **9**. Compound **22** was prepared in a similar manner where an appropriate 2-iodo-hetrocycle **21** was coupled with boronic acid **9** under standard Suzuki–Miyura conditions and finally the Boc protecting group was removed with TFA (Scheme 2).

Compound **14** was prepared from **8b** where it was first treated with alkyl bromide and then the ester was hydrolyzed with LiOH to give the corresponding carboxylic acid derivative **13** which was then coupled with **9a** (Scheme 3). The carboxylic acid on **14** was then reacted with morpholine and HBTU to give compound **15**. Treating intermediate **8b** with 2-bromoethanol gave alcohol 12 that was then coupled with boronic acid **9a** and subsequent removal of Boc protecting group with TFA gave compound **17**. Compound **20** was prepared from alcohol **12** which was first converted to the mesylate and then displaced by an amine to give intermediate **19** which was subsequently coupled with **9a** followed by the removal of the Boc protecting group.

2-Acetylene-indole **24** was prepared by coupling 2-iodoindole **7** with TIPS-acetylene using Sonogashira coupling condition with $PdCl_2(PPh_3)_2$ and CuI, and then the phenylsulfonyl protecting group was removed with TBAF (Scheme 4). A second Sonogashira coupling of intermediate **24** with **7** followed by removal of the phenylsulfonyl group gave compound **25**. Treating **25** with MeI gave a mixture of mono-methylated compound **26a** and dimethylated compound **26b**. Attempts were made to reduce the acetylene linker of compound **25** as a route to synthesize **27b**; however, we were only able to isolate the mono-brominated compound **27a**, which was nonetheless useful for our SAR study (Scheme 5). Compound

27b was finally synthesized by cross-coupling two molecules of 6-bromo-1*H*-indole-2-carbaldehyde using titanium tetrachloride and zinc dust, which also gave **28a** as a bi-product. Compound **27c** was prepared from alcohol **29** where it was first converted to Wittig salt **30** (Scheme 5). Compound **30** was then coupled with the corresponding aldehyde to give **31** and finally the phenylsulfonyl protecting groups were removed with Cs_2CO_3 to give the desired adduct **27c**. The double bond of compound **27c** was reduced by hydrogenation over Pt/C to give compound **28b**.

Symmetrical bis-indoles **33a** and **33b** were prepared by double Suzuki–Miyura reaction of boronic acid **9** with aryl di-halide **32** followed by the removal of the Boc protecting group with TFA (Scheme 6). In order to prepare unsymmetrical bis-indoles **33c**–**e**, aryl di-halide was first coupled with 1 equiv of boronic acid **9** to give intermediate **34** which was consequently coupled with a different boronic acid **9a** and finally the Boc group was cleaved with TFA to give the desired compounds.

2.2. Biological results and SAR studies

All compounds prepared were screened against in an in vitro enzyme assay to assess MRSA PK inhibitory activity. Cellular antimicrobial activity was evaluated for ability to fully inhibit the growth of *S. aureus* ATCC 29213 in culture (determining minimum fully inhibitory concentration (MIC) values) (Tables 1–3). Selectivity for the MRSA PK over the four human isoforms was confirmed for a selected series of compounds (Table 4). Cytotoxicity was evaluated for selected compounds with HEK 293 cells and they were found not to be significantly cytotoxic at concentration up to 100 μg/ml (Table 5).

2.2.1. SAR for in vitro inhibition of MRSA PK—To further improve and optimize biological activity we set out to develop SAR for both MRSA PK enzyme and the cellular antibacterial activity in parallel. For the purpose of clarity we will discuss the SAR derived for inhibition of the MRSA PK enzyme separately from the cellular antibacterial activity.

We systematically evaluated the effect of substitution on bis-indole scaffold (Table 1), replacing one indole ring with a number of heterocycles (Table 2) and modifying the central linking moiety (Table 3).

The directly linked 6,6′-dibromo-1*H*,1′*H*-2,2′-biindole (**10b**) was prepared and we were pleased to note that it was a potent inhibitor of the MRSA PK enzyme with an IC_{50} of 7.0 nM and that it also gave an MIC against *S. aureus* of 16 μg/ml (Table 1). To evaluate the role of the two bromines, the mono-brominated compound **10a** was prepared and was found to be about threefold less active. However, the asymmetrically substituted 6,5^0-dibrominated com-pound **10c** was more potent with IC_{50} of 2.2 nM. It was found that the 5′-bromine could be substituted with chloro (**10d**), fluoro (**10e**), methoxy (**10f**) or even with a relatively bulky group such as phenyl (**10g**) without significant loss of potency suggesting that there are still some room in the binding pocket which might be further exploited to improve activity.

We next prepared the 5′-monobrominated bis-indole analog (**10h**) and noted a significant drop in activity. This suggested that at least one bromine atom in the 6-position of one of the

indole moieties was critical for activity. On the other hand, however, the 5,5′ dibromo bis-indole (**10i**) was found to be very potent with an IC_{50} of 3 nM. It is not clear why **10h** was not active whereas **10i** is potent but one possible explanation could be that one of the bromines of **10i** is oriented towards the interior of the binding pocket and the other bromine is facing outwards, thus placing the indole NH towards the interior to provide a necessary hydrogen bonding with Ser362. Mysteriously, the 5,6 dibromo compound **10j** was not potent whereas the 5,6,6′- and 5,6,5′-tribromo compounds **10k** and **10m** and tetrabromo bis-indole **10l** were found to be very potent. The activity of these relatively large molecules was in keeping with there being still more space in the binding pocket to be exploited (discussed later, Table 3).

Next we investigated the effect of substitution on the NH of in-dole. The mono-*N*-methyl bis-indole **12a** was very active with an IC_{50} of 1.0 nM whereas the isomeric mono-*N*-methyl bis-indole **12b** was almost inactive. This further confirmed that the presence of a bromine atom at the 6-position relative to the indole NH is important for activity. It appears that the bromine and NH of 6-bromo-indole fragment binds very tightly to the pocket and there is no room for further substitution in that region of the molecule. The methyl group in **12a** is most likely oriented towards the outside (water side) of the binding site and that explains why compounds **12c, 14, 15, 17, 20a** and **20b** with bulky groups attached to the nitrogen atom of the 5-bromo indole are still very potent. There was no further improvement in activity by either introducing polar (**12c, 15, 17** and **20b**), acidic (**14**) or basic (**20a**) groups at NH of the second indole.

Our next step was to investigate whether both indoles are critical for binding or if one can be replaced with other heterocycles. Keeping the 6-bromoindole element constant and replacing the second indole with benzothiazole (**22a**) or benzofuran (**22b**) led to a 5–8-fold decrease in activity (compared to **10a**). However, the benzothiophene derivative (22c) is slightly more potent than **10a** suggesting that one indole can indeed be replaced with benzo-thiophene. In the cases where we kept one 5-bromoindole constant and replaced the other indole with benzothiazole (**22d**), benzofuran (**22e**) or benzothiophene (**22f**), there was a significant drop in activity as expected from earlier observation. Nevertheless, 6-bromothiazole analog (**22g**) was about fivefold more potent than **10a** and comparable in potency to 5,5′-dibromo-bis-indole **10i**, and hence might have a similar mode of binding.

Next we investigated whether a spacer moiety could be placed between the two indoles. Compound **25a**, with an acetylene linker, was found to be threefold more potent than direct linked compound (**10a**) indicating that the binding pocket has more linear space. Next we made the 6,6′-dibromo analog (**25b**) and 6,5′-dibromo analog (**25c**) with an acetylene linker, but there was no significant improvement in potency. One indole NH could be methylated (**26a**) without significant loss in activity, but as expected, methylation of both NH (**26b**) led to a compound that was essentially inactive. Surprisingly **27a** and **27b** with an ethylene linker was almost equipotent as the corresponding acetylene linker. We had anticipated a loss in potency as the two indoles should adopt a linear staggered conformation with ethylene linker that should displace one of the indoles within the binding pocket but evidently this is not detrimental. More interestingly, compounds (**28a** and **28b**) with the fully

saturated ethane linker moiety were still very potent, both with IC_{50} of 6 nM. Compounds **28a** and **28b** presumably are able to orient in a staggered linear and planer conformation similar to **27a** and **27b** to bind in the flat lipophilic pocket similar to the natural product **4**. In order to make the spacer slightly longer we synthesized 1,4-phenyl linked compound **33a**. There was a significant drop in activity observed for **33a** (as com-pared to **25a**) and modelling suggests that it might be now too long to fit in the binding pocket. Subsequently, we made a slightly shorter analog **33c** (by removing one bromine atom) and this compound was found to be very potent (IC_{50} of 5.6 nM). Hence it appears that **33c** is occupying most of the binding pocket and it defines the breadth of the site.

It was noted that if **33** was modelled into the binding site, the central ring was flanked above and below by the His-365 residues. Considering that these interactions might lead to some potential for charge transfer interaction, we investigated the effect of placing an electron-withdrawing group (**33d**) or an electron-releasing group (**33e**) on the phenyl linker. However both **33d** and **33e** were about 2–3 fold more potent than **33c** so it is not clear if such an interaction exists. 1,3-Phenyl-linked (**33f**) and 2,5-thiophene linked (**33b**) were essentially inactive which demonstrates that both indoles have to be able to adopt an essentially linear attitude for significant binding.

2.2.2. SAR for anti-*S. aureus* activity—For those tested, compounds with low potency against MRSA PK (IC_{50} >100 nM) were also poorly effective (MIC >64 μg/ml) against *S. aureus* ATCC95923. However while many potent inhibitors of MRSA PK gave low MICs (e.g., compound **10d**), other potent ana-logs had high MICs. This suggested that issues such as solubility, cellular penetration or active export from the bacterial cells might impair full translation of activity. The MIC of the initial lead compound **10b** was similar to that reported for *cis*-3–4-dihydroham-acanthin B (**4**). However, compound **10a** with one less bromine was about eightfold more potent than **10b** despite it being three-fold less potent in MRSA PK enzyme assay. Direct linked bis-indole compounds 6,5′-dibromo (**10c**), 6-bromo-5-chloro (**10d**) and 6-bromo-5-fluoro (**10e**) were most potent with MICs of 0.3 μg/ml. However, although 5,5′-dibromo derivative **10i** and tetrabromo **10l** were equipotent in vitro compared to **10b**–**10e**, they had much higher MICs (>64 μg/ml). Both tribromo substituted compounds **10k** and **10m** showed excellent MICs.

Many of these compounds had limited solubility that might limit ability to access and to penetrate cells and therefore a number of potentially solubilizing groups were installed on one of the indole NHs (see compounds **12c, 14, 15, 17, 20a** and **20b**). However, although all these compounds were potent inhibitors of MRSA PK, only the hydroxyethyl analog **17** and piperazinylethyl derivative **20a** showed reasonable MICs. Hence, it appears that there was no clear correlation between the MIC and polarity. In the cases where one indole was replaced with a heterocycle (**22a**–**g**), none gave significant MIC despite being potent in the enzyme assay (e.g., compound **22g**). Of all the compounds made with a spacer between the two indoles only **25c** and **33d** gave significant MIC values further exemplifying the lack of correlation of enzymatic activity and anti-bacterial activity. Of all the phenyl-linked compounds made, only **33d** bearing an electron-with drawing group was MIC active.

2.2.3. Evaluation of the effect of verapamil on the MIC of selected PK inhibitors—Considering the divergent anti-*S. aureus* activity observed among very close structural analogs such as **10b** and **10l** compared to **10c, 10d** or **10e,** in spite of similar IC_{50}s for inhibition of MRSA PK, it seemed unlikely that cell penetrability or solubility could explain these discrepancy. One possibility is that compounds such as **10b** may be substrates for putative bacterial efflux systems, such as has been proposed to impart resistance of many bacteria to antibiotics.[9] The antihypertensive drug, verapamil has been shown to be an inhibitor the ABC class of pumps in Gram-positive bacteria such as *Staphylococci* and verapamil has been demonstrated to inhibit efflux pumps in *S. aureus*.[10,11] We therefore evaluated the effect of verapamil on the observed MIC for several key compounds. MICs were determined for *S. aureus* ATCC92953 in the absence and presence of verapamil (200 mg/L, a concentration previously reported as an optimal sub-inhibitory concentration[11]). Vancomycin was used as control and gave the same MIC (2 μg/ml) in presence and absence of verapamil as did compound **10d** (MIC = 0.5 μg/ml). However, the MIC of **10l** was shifted at least fourfold (from >64 to 16 μg/ml) by verapamil while the MIC of **10b** was dramatically shifted, 32-fold (from 16 to 0.5 μg/ml) (Fig. 3).

2.2.4. Evaluation of efficacy of selected MRSA PK inhibitors against MRSA—To further investigate the antibacterial properties of this class of PK inhibitors, the three of the most potent compounds (**10c**, **10d** and **10e**) from the SAR study were tested in vitro for their antibacterial activities against MRSA strain MW2 (USA400) with vancomycin as positive control. In each case an MIC of 0.5 μg/ml was observed while vancomycin gave an MIC of 2 μg/ml (Fig. 4).

2.2.5. Evaluation of MRSA PK inhibitors in bacteria pyruvate kinase deficient *S. aureus* cells—To implicate **10d**-mediated inhibition of *S. aureus* PK as the direct cause of bacterial killing, we constructed a *S. aureus* LAC Δ*pyk*::Erm^R mutant. Essentially, we replaced the *pyk* gene on the chromosome of *S. aureus* with an Erm^R cassette using a standard allelic exchange procedure as previously described.[12] Previous work has described PK as an essential enzyme required for growth.[2,3] In each of these previous studies however, the growth medium used contained primarily glycolytic carbon sources such as glucose (G). By designing media lacking carbohydrates but replete with pyruvate (P), PK becomes dispensable and a mutant can therefore be made that has relatively no growth defect under these specific conditions (Fig. 5 shows that in TSB-G+P, the Δ*pyk* and WT grow identically in the absence of **10d**). Initial characterization of the mutant revealed that it exhibits an aerobic growth defect when grown on Brain–Heart Infusion Broth (BHI) but not on Tryptic Soy Broth (TSB) without glucose, provided the medium is supplemented with 1% sodium pyruvate (TSB-G+P) (Fig. 5). The growth defect exhibited by *S. aureus* Δ*pyk*::Erm^R on BHI was reversible via complementation using a plasmid expressing *pyk* from a constitutive promoter (data not shown). These data indicate that the metabolic activity of PK is required for aerobic growth of *S. aureus* on glycolytic carbon sources (e.g., BHI) but not certain combinations of gluconeogenic carbon sources (e.g., TSB-G+P).

We next examined the bacteriostatic and bactericidal effects of **10d** on wild type (WT) *S. aureus* as well as the Δ*pyk*::Erm^R. We found that, while **10d** was highly bactericidal to WT

S. aureus, the drug merely acted statically on the Δ*pyk*::Erm^R mutant in BHI medium (Fig. 5A). The Δ*pyk*::Erm^R mutant grows slower than WT in BHI medium and to test whether this had any effect on **10d** toxicity, we tested the compound's effect in media in which the mutant has no growth defect. As in BHI, **10d** was unable to kill the Δ*pyk*::Erm^R mutant in TSB-G+P as it did WT *S. aureus* (Fig. 5B). Thus, while **10d** efficiently eliminated growth of both WT and Δ*pyk*::Erm^R *S. aureus* (no significant difference in 10d MIC for either strain), the bactericidal activity of this compound is completely dependent on PK activity (Fig. 5).

2.2.6. Selectivity and cytotoxicity—For selected compounds selectivity for MRSA PK relative to the mammalian PK isoforms M1, M2, R and L (Table 4) was determined using enzymes and methods as previously described.[4,8] Those compounds tested for selectivity generally showed no significant inhibition at the highest concentration tested (10 μM) and only **20b** showed inhibition of a mammalian PK isoform of greater than 50% (51% for the R isoform). Cytotoxicity was evaluated for selected compounds with HEK 293 cells as previously described[4,8] and they were found not to be significantly cytotoxic at concentration up to 100 μg/ml (Table 5).

3. Conclusions

Based on observations from the published crystal structure of a naturally occurring marine alkaloid **4** bound to the tetrameric MRSA PK enzyme, a novel series of bis-indoles were [stat] designed and prepared and shown to be very potent inhibitors of MRSA pyruvate kinase. Compounds such as **10c, 10d, 10e, 10k, 10m** and **12a** are among the most potent inhibitors of this enzyme identified to this date with IC_{50}s of 1–3 nM. Numerous analogs were synthesized in order to define structure–activity relationships and to further optimize the physiochemical properties for effective inhibition of bacterial growth. Lead compounds were very selective for the bacterial PK over the human isoforms. Although there was, overall, a poor correlation between the in vitro enzyme inhibitory activity and anti-bacterial activity against *S. aureus* ATCC 29213, some compounds showed excellent MIC values of as low as 0.3 μg/ml while lacking significant mammalian cytotoxicity.

Considering that this lack of correlation might be due (at least in part) to selective active efflux from the bacteria, studies were carried out on the effect of the known efflux inhibitor, verapamil, on the MIC values of selected close structural analogs, **10a, 10d** and **10l**. These studies revealed a pronounced enhancement of MIC values for **10a** and **10l** but not for **10d** (or vancomycin control) indicating that at least for these key compounds the attenuated antibacterial activity is due to active efflux mechanisms.

Compounds **10c, 10d** and **10e** were further evaluated for efficacy against MRSA strain MW2 where MICs (0.5 μg/ml) similar to those obtained for *S. aureus* ATCC 29213 were observed. In previous studies on MRSA pyruvate kinase inhibitors[4,8] potency against *S. aureus* ATCC 29213 was found to be representative of potency against MRSA strains and thus we infer that this will also be the case for this class of compounds.

In order to gain further insight into the mechanism of action of these compounds on *S. aureus* we evaluated the effects of the compound **10d** on a *S. aureus* mutant where the gene

for pyruvate kinase (*Pyk*) is knocked out. These cells are viable when cultured in a medium lacking usable carbohydrate and additionally supplemented with pyruvate. These studies showed that the bacteriostatic and bactericidal effects of **10d** on *S. aureus* occur by different mechanisms. The bacteriostatic effects of **10d** are *pyk*-independent while the bactericidal effects of 10d are *pyk*-independant. The bactericidal mechanism of 10d does not require PK metabolic activity (**10d** is bactericidal to *S. aureus* on TSB-G+P, a growth media where *pyk*::Erm^R exhibits no growth defects). This supports the model of pyruvate kinase as a highly interactive protein and the expectation that pyruvate kinase inhibitors of this class may yield effective anti-MRSA compounds with low potential for development of resistance.

The lead compounds (**10c, 10d, 10e, 10k** and **10m**) with best MIC values are currently being evaluated to determine in vivo absorption and tissue exposure in preparation for evaluation in in vivo animal infection models. These studies will be reported elsewhere.

4. Experimental section

4.1 Chemistry

4.1.1. General synthesis methods—^{1}H and ^{13}C NMR spectra were recorded with either Bruker Avance II™ 600 MHz, Bruker Avance III™ 500 MHz or Bruker Avance III™ 400 MHz. Processing of the spectra was performed with MestRec™ software. The high-resolution mass spectra were recorded either in positive or negative ion-mode with an ESI or multimode ESI/APCI ion source on an Agilent™ 6210 Time-of-Flight LC/MS mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on aluminum plates pre-coated with silica gel 60F-254 as the absorbent. The developed plates were air-dried, exposed to UV light and/or dipped in $KMnO_4$ solution and heated. Column chromatography was performed with silica gel 60 (230–400 mesh). Automated flash chromatography was carried out on Biotage Isolera Flash Purification Systems using commercial 50 μm silica gel cartridges. Purity (>95%) for all final compounds was confirmed by analytical reverse-phase HPLC utilizing a Dikma Technologies™ Inspire® C18 reverse-phase analytical column (4.6 × 150 mm) using ACN and H_2O with 0.1% formic acid as eluent. All HPLC purifications were carried out using an Agilent™ C18 reverse-phase preparatory column (21.2 × 250 mm) using ACN and H_2O with 0.1% formic acid as eluent.

4.1.1.1. General procedure for the synthesis of 1-(phenylsulfonyl)-1*H*-indole (6).: To a stirred solution of an appropriate indole (1 mmol) in THF (25 ml) at 0 °C was added NaH (60% in oil, 1.2 mmol) gradually. After stirring at room temperature for 10 min benzenesulphonyl chloride (1.2 mmol) was added and the mixture was further stirred for 2 h. The reaction was quenched with saturated ammonium chloride solution and extracted with EtOAc (2 × 50 ml). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by automated flash chromatography using EtOAc and hexanes as eluents to give the desired product.

4.1.1.2. General procedure for the synthesis of 2-iodo-(phenylsulfonyl)-1*H*-indole (7).: To a stirred solution of **6** (1 mmol) in anhydrous THF (10 ml) at −78 °C was added a solution of LDA (1.5 mmol) in THF (5 ml). The mixture was stirred for at −78 °C for 100 min and then warmed to 0 °C for 30 min. The solution was re-cooled to −78 °C and then either a solution of 1,2-diiodo ethane or molecular iodine (1.5 mmol) in THF (10 ml) was added. The reaction mixture was stirred at 0 °C for 15 min and then allowed for warm to room temperature for 1 h. The reaction was quenched with saturated NH_4Cl solution and extracted with EtOAc (2 × 50 ml). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by automated flash chromatography using EtOAc and hexanes as eluents to give the desired product.

4.1.1.3. 6-Bromo-2-iodo-1-(phenylsulfonyl)-1H-indole (7a).: Yield = 53%, white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.16 (d, J = 9.0 Hz, 1H), 7.87 (d, J = 7.6 Hz, 2H), 7.58 (t, J = 7.5 Hz, 1H), 7.5 (d, J = 1.6 Hz, 1H), 7.46 (t, J = 7.8 Hz, 2H), 7.37 (dd, J = 9.0, 1.9 Hz, 1H), 6.93 (s, 1H). HRMS calcd for $(C_{14}H_9BrINO_2S–H)^-$ 460.8582; found 460.85998.

4.1.1.4. 5-Bromo-2-iodo-1-(phenylsulfonyl)-1*H*-indole (7b).: Yield = 54%, white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15 (d, J = 9.0 Hz, 1H), 7.87 (d, J = 8.5 Hz, 2H), 7.56 (t, J = 8.1 Hz, 1H), 7.53 (d, J = 1.7 Hz, 1H), 7.44 (t, J = 7.5 Hz, 2H), 7.37 (dd, J = 2.0, 9.0 Hz, 1H), 6.91 (s, 1H).

4.1.1.5. 5-Chloro-2-iodo-1-(phenylsulfonyl)-1*H*-indole (7c).: Yield = 75%, white Solid. 1H NMR ($CDCl_3$, 400 MHz): δ 8.21 (d, J = 9.0 Hz, 1H), 7.84–7.90 (m, 2H), 7.55–7.61 (m, 1H), 7.46 (dt, J = 7.4, 1.8 Hz, 2H), 7.38 (d, J = 1.9 Hz, 1H), 7.24 (dd, J = 9.0, 2.2 Hz, 1H), 6.93 (d, J = 0.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): 138.12, 136.95, 134.44, 132.87, 129.82, 129.42, 127.31, 126.80, 125.21, 123.43, 119.36, 116.55 ppm. HRMS calcd for $(C_{14}H_9ClINO_2S–H)^-$ 416.9087; found 416.9094.

4.1.1.6. 5-Fluoro-2-iodo-1-(phenylsulfonyl)-1*H*-indole (7d).: Yield = 94%, white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.23 (dd, J = 9.2, 4.4 Hz, 1H), 7.84–7.89 (m, 2H), 7.54–7.61 (m, 1H), 7.42–7.48 (m, 2H), 6.97–7.08 (m, 2H), 6.95 (d, J = 0.5 Hz, 1H). HRMS calcd for $(C_{14}H_9FINO_2S–H)^-$ 400.9383; found 400.9392.

4.1.1.7. General procedure for the synthesis of substituted 2-iodo-1*H*-indole (8).: To a stirred solution of compound 7 (1 mmol) in THF (20 ml) at room temperature was added a solution of TBAF (1 ml, 1 M in THF, 1 mmol). The mixture was stirred at ambient temperature for 5 h and then partitioned between EtOAc (100 ml) and H_2O (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by automated flash chromatography using EtOAc and hexanes as eluents to give compound **8**.

4.1.1.8. 6-Bromo-2-iodo-1*H*-indole (8a).: Yield = 48%, white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.07 (br s, 1H), 7.46–7.50 (m, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.19 (dd, J = 8.4, 1.7 Hz, 1H), 6.69 (dd, J = 2.0, 0.9 Hz, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz): 139.38, 128.33,

122.17, 120.23, 114.01, 112.90, 110.84, 79.73. HRMS calcd for $(C_8H_5BrIN–H)^-$ 320.8650; found 320.8658.

4.1.1.9. 5-Bromo-2-iodo-1*H*-indole (8b).: Yield = 92%, white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.9 (br s, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.28 (d, J = 8.6 Hz, 1H), 7.15 (dd, J = 1.9, 8.6 Hz, 1H), 6.6 (s, 1H).

4.1.1.10. 5-Chloro-2-iodo-1*H*-indole (8c).: Yield = 96%, pale brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 (br s, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.24 (d, J = 8.7 Hz, 1H), 7.09 (dd, J = 8.6, 2.0, 1H), 6.67 (d, J = 1.2 Hz, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 137.16, 130.31, 123.94, 121.09, 117.68, 111.88, 110.33, 80.64. HRMS calcd for $(C_8H_5ClIN–H)^-$ 276.9155; found 276.9158.

4.1.1.11. 5-Fluoro-2-iodo-1*H*-indole (8d).: Yield = 85%, white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.08 (br s, 1H), 7.22–7.25 (m, 1H), 7.19 (dd, J = 9.4, 2.5 Hz, 1H), 6.89 (td, J = 9.1, 2.5 Hz, 1H), 6.68 (dd, J = 2.0, 0.8 Hz, 1H).

4.1.1.12. 5,6-Dibromo-2-iodo-1*H*-indole.: Yield = 89%, white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09 (br s, 1H), 7.80 (s, 1H), 7.69 (d, J = 0.7 Hz, 1H), 6.64 (dd, J = 0.8, 2.3 Hz, 1H).

4.1.1.13. General procedure for the synthesis of substituted 2-iodo-1-methyl-1*H*-indole (11).: A mixture of an appropriate 2-iodo-1*H*-indole **8** (1 mmol), K_2CO_3 (2 mmol) and methyl iodide (1.5 mmol) in DMF was stirred at room temperature for 3 d. The reaction mixture was partitioned between EtOAc (100 ml) and H_2O (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by automated flash chromatography using EtOAc and hexanes as eluents to afford compound **11**.

4.1.1.14. 6-Bromo-2-iodo-1-methyl-1*H*-indole (11a).: Yield = 97%, white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.46–7.47 (m, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.17 (dd, J = 8.4, 1.7 Hz, 1H), 6.76 (d, J = 0.8 Hz, 1H), 3.72 (s, 3H).

4.1.1.15. 5-Bromo-2-iodo-1-methyl-1*H*-indole (11b).: Yield = 99%, white solid. ^{1}HNMR($CDCl_3$, 500 MHz): δ 7.63 (d, J = 1.6 Hz, 1H), 7.22 (dd, J = 8.7, 1.8 Hz, 1H), 7.16 (d, J = 8.7 Hz, 1H), 6.72 (s, 1H), 3.73 (s, 3H). HRMS calcd for (C_9H_7BrIN) 334.8807; found 334.8780.

4.1.1.16. Synthesis of 5-bromo-2-iodo-1-(methoxymethyl)-1*H*-indole (11c).: To a stirred slurry of NaH (22 mg, 60% in oil, 0.94 mmol) in THF (5 ml) and DMF (1 ml) at 0 °C was added a solution of **8b** (200 mg, 0.63 mmol) in THF (5 ml). After stirring for 1 h methoxymethyliodide (64 μL, 0.75 mmol) was added and the mixture was further stirred for 2 h. The reaction mixture was partitioned between Et2O (100 ml) and H_2O (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na2-SO4 and concentrated. The residue was purified by automated flash chromatography using EtOAc and hexanes as eluents to afford **11c** (178 mg, 77%) as yellow solid. ^{1}H NMR (DMSO-d_6,

500 MHz): δ 7.70 (d, J = 1.8 Hz, 1H), 7.60 (d, J = 8.7 Hz, 1H), 7.26 (dd, J = 8.7, 1.9 Hz, 1H), 6.85 (s, 1H), 5.52 (s, 2H), 3.19 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): 136.66, 131.25, 124.32, 121.35, 112.96, 112.51, 112.31, 88.25, 76.62, 55.46. HRMS calcd for ($C_{10}H_9BrINO$) 364.8192; found 364.8923.

4.1.1.17. Synthesis of 2-(5-bromo-2-iodo-1*H*-indol-1yl)acetic acid (13).: To a stirred slurry of NaH (144 mg, 60% in oil, 6.0 mmol) in DMF (5 ml) at 0 °C was added a solution of **8b** (960 mg, 3.0 mmol) in DMF (15 ml). The mixture was stirred at 0 °C for 30 min and then at rt for an additional 30 min. Ethyl 2-iodoacetate (225 μL, 3.6 mmol) was added and the mixture was stirred at rt for 16 h. The reaction mixture was diluted with EtOAc and then washed with 1 M HCl followed by brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was dissolved in a mixture of THF and LiOH solution at 0 °C and stirred at rt until the completion of the reaction as indicated by TLC. The reaction mixture was acidified and extracted with EtOAc. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by automated flash chromatography to give white solid (1.01 g, 89%). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.17 (br s, 1H), 7.69 (d, J = 1.8 Hz, 1H), 7.50 (d, J = 8.8 Hz, 1H), 7.21 (dd, J = 8.7, 2.0 Hz, 1H), 6.81 (s, 1H), 5.0 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): 169.46, 136.74, 130.82, 123.92, 121.11, 112.36, 112.26, 111.22, 89.24, 48.26. HRMS calcd for ($C_{10}H_7BrINO_2$–H)$^-$ 378.8705; found 378.8691.

4.1.1.18. Synthesis of 2-(5-bromo-2-iodo-1*H*-indol-1-yl)ethanol (16).: To a solution of **8b** (38 mg, 0.11 mmol) in DMF (1 ml) was added bromoethanol (14 mg, 0.12 mmol) and K_2CO_3 (49 mg, 0.36 mmol). The reaction heated at 180 °C with lwave for 30 min and then partitioned between EtOAc and H_2O. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by automated flash chromatography using EtOAc and hexanes as eluents to afford **16** (24 mg, 59%) as white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.65 (s, 1H), 7.28 (d, J = 7.7 Hz, 1H), 7.23 (dd, J = 8.7, 1.8 Hz, 1H), 6.75 (s, 1H), 4.32 (t, J = 5.6 Hz, 2H), 3.9–3.97 (m, 2H).

4.1.1.19. Synthesis of 2-(5-bromo-2-iodo-1*H*-indol-1-yl)ethyl-methanesulfonate (18).: To a stirred solution of **16** (3.0 g, 8.2 mmol) in DCM (20 ml) at 0 °C was added Et_3N (1.8 ml, 12.4 mmol) and methanesulfonyl chloride (0.64 ml, 8.2 mmol). The reaction mixture was stirred at rt for 1 h and then quenched by the addition of ice. The reaction mixture was extracted into DCM (3 × 10 ml) and the combined extract was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give essentially pure compound **14** as brown solid (2.98 g, 82%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.66 (m, 1H), 7.24–7.28 (m, 2H), 6.78 (s, 1H), 4.45–4.55 (m, 4H), 2.71 (s, 3H). HRMS calcd for ($C_{11}H_{11}BrINO_3S$) 442.8688; found 442.8686.

4.1.1.20. General procedure for the synthesis of 2-iodoindole derivative (19).: To a stirred solution of the intermediate **18** (1 mmol) in DMF (3 ml) was added K_2CO_3 (1.5 mmol) and the corresponding amine (3 ml). The mixture was heated at 50 °C for 12–20 h and then partitioned between EtOAc and H_2O. The organic phase was washed with saturated NH_4Cl, brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by

automated flash chromatography using EtOAc and hexanes as eluents to afford the desired product **19**.

4.1.1.21. 5-Bromo-2-iodo-1-(2-(4-methylpiperazin-1-yl)ethyl)-1*H*-indole (19a).: Yield = 89%, white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.61–7.64 (m, 1H), 7.19–7.21 (m, 2H), 6.69 (s, 1H), 4.20–4.30 (m, 2H), 2.35–2.65 (m, 10H), 2.29 (s, 3H). ^{13}C NMR (125 MHz): 136.02, 131.22, 124.66, 122.06, 113.30, 111.53, 111.01, 103.91, 57.18, 55.01, 53.46, 46.14, 45.21. HRMS calcd for ($C_{15}H_{19}BrIN_3$) 446.9807; found 446.9814.

4.1.1.22. 4-(2-(5-Bromo-2-iodo-1*H*-indol-1-yl)ethyl)morpholine (19b).: Yield = 55%, brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.65–7.64 (m, 1H), 7.26–7.22 (m, 2H), 6.72 (s, 1H), 4.27 (t, J = 7.2 Hz, 2H), 3.70 (br s, 4H), 2.63 (t, J = 7.0 Hz, 2H), 2.53 (br s, 4H).

4.1.1.23. General procedure for the synthesis of compounds 10, 12, 14, 17, 20, 22, 33 and 24.: A solution of boronic acid **9** (1 mmol), iodo-heterocycle (**8, 11, 21, 32** or **34**) (1 mmol), Na_2CO_3 (1 M aqueous solution, 3.5 mmol) in ACN (5 ml) was purged with argon for 10 min followed by the addition of $Pd(PPh_3)_2Cl_2$ catalyst (10 mol %). The mixture was heated in a sealed tube with μwave at 110 °C until all the staring material was consumed as indicated by TLC (typically in about 40–60 min). The reaction mixture was partitioned between EtOAc (100 ml) and H_2O (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4 and concentrated. The residue was taken up in DCM (10 ml) and then TFA (1 ml) was added. After stirring at room temperature for 2 h, solvent was removed and the crude product was purified by automated flash chromatography using either EtOAc and hex-anes or MeOH and DCM as eluents to give the desired adduct.

4.1.1.24. 6-Bromo-1*H*,1′*H*-2,2′-biindole (10a).: Compound **10a** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and *tert*-butyl 2-iodo-1*H*-indole-1-carboxylate.

Yield = 35%, pink solid. Mp = 199–201 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.71 (s, 1H), 11.59 (s, 1H), 7.51–7.59 (m, 3H), 7.41 (d, J = 8.1 Hz, 1H), 7.09–7.16 (m, 2H), 7.02 (t, J = 7.4 Hz, 1H), 6.92–6.95 (m, 2H). ^{13}C NMR (DMSO-d_6, 150 MHz): 137.04, 136.85, 132.34, 130.69, 128.20, 127.42, 122.21, 121.92, 121.65, 120.15, 119.48, 114.14, 113.35, 111.13, 98.79, 98.37. HRMS calcd for ($C_{16}H_{11}BrN_2$ H) 310.0106; found 310.0107.

4.1.1.25. 6,6′-Dibromo-1*H*,1′*H*-2,2′-biindole (10b).: Compound **10b** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 6-bromo-2-iodo-1*H*-indole (**8a**).

Yield = 63%, white solid. Mp = 266–267 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.77 (s, 2H), 7.56 (s, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.15 (dd, J = 1.6 Hz, zH8.4 Hz, 2H), 6.95 (d, J = 1.1 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 137.8 (2C), 131.8 (2C), 127.4 (2C), 122.4 (2C), 121.9 (2C), 114.4 (2C), 113.5 (2C), 99.0 (2C). HRMS calcd for ($C_{16}H_{10}Br_2N_2$ H) 388.9118, found 388.9123.

4.1.1.26. 5,6-Bibromo-1*H*,1′H-2,2′-biiindole (10c).: Compound **10c** was prepared from (6-bromo-1-(tert-butoxycarbonyl)-1H-indol-2-yl)boronic acid (**9a**) and 5-bromo-2-iodo-1H-indole (**8b**).

Yield = 45%, pale brown solid. Mp = 270–272 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.82 (s, 1H), 11.78 (s, 1H), 7.79 (s, 1H), 7.53–7.57 (m, 2H), 7.37 (d, J = 8.5 Hz, 1H), 7.23 (d, J = 8.6 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 6.97 (s, 1H), 6.93 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): 137.86, 135.68, 132.28, 131.74, 130.26, 127.38, 124.30, 122.43, 122.28, 121.88, 114.47, 113.58, 113.01, 111.98, 99.11, 98.43. HRMS calcd for $(C_{16}H_{10}Br_2N_2–H)^-$ 387.9211; found 387.9227.

4.1.1.27. 6′-Bromo-5-chloro-1*H*,1′*H*-2,2′-biindole (10d).: Compound **10d** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 5-chloro-2-iodo-1*H*-indole (**8c**).

Yield = 60%, white solid. Mp = 257–259 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.80 (s, 1H), 11.77 (s, 1H), 7.64 (d, J = 1.8 Hz, 1H), 7.52–7.55 (m, 2H), 7.40 (d, J = 8.6 Hz, 1H), 7.14 (dd, J = 8.4, 1.7 Hz, 1H), 7.11 (dd, J = 8.6, 2.0 Hz, 1H), 6.95 (d, J = 1.5 Hz, 1H), 6.92 (d, J = 1.5 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): 137.83, 135.44, 132.44, 131.77, 129.51, 127.36, 123.99, 122.41, 121.89, 121.77, 119.23, 114.43, 113.55, 112.54, 99.04, 98.53. HRMS calcd for $(C_{16}H_{10}BrClN_2–H)^-$ 343.9716; found 343.9727.

4.1.1.28. 6′-Bromo-5-fluoro-1*H*,1′*H*-2,2′-biindole (10e).: Compound **10e** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 5-fluoro-2-iodo-1*H*-indole (**8d**).

Yield = 61%, white solid. Mp = 246–248 °C. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.73 (s, 1H), 11.68 (s, 1H), 7.51–7.56 (m, 2H), 7.38 J = 8.4, 1.8 Hz, 1H), 6.90–6.97 (m, 3H). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 157.68 (d, J = 231.7 Hz), 138.26, 134.13, 133.10, 132.42, 129.06 (d, J = 10.6 Hz), 127.86, 122.87, 122.31, 112.48 (d, J = 9.8 Hz), 114.82, 114.02, 110.46 (d, J = 26.1 Hz), 105.13 (d, J = 23.4 Hz), 99.48 (d, J = 4.7 Hz), 99.27. HRMS calcd for $(C_{16}H_{10}BrFN_2–H)^-$ 328.0011; found 328.0019.

4.1.1.29. 6′-Bromo-5-methoxy-1*H*,1′*H*-2,2′-biindole (10f).: Compound **10f** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and *tert*-butyl 2-iodo-5-methoxy-1*H*-indole-1-carboxylate.

Yield = 16%, pale brown solid. Mp = 240–241 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.67 (s, 1H), 11.43 (s, 1H), 7.50–7.54 (m, 2H), 7.28 (d, J = 8.7 Hz, 1H), 7.12 (dd, J = 8.4, 1.7 Hz, 1H), 7.07 (d, J = 2.4 Hz, 1H), 6.89 (s, 1H), 6.84 (s, 1H), 6.76 (dd, J = 8.7, 2.4 Hz, 1H), 3.77 (s, 3H). ^{13}C NMR (DMSO-d_6, 150 MHz): 153.68, 137.69, 132.51, 132.10, 131.19, 128.73, 127.50, 122.24, 121.61, 113.99, 113.40, 112.20, 111.75, 101.67, 98.80, 98.17, 55.30. HRMS calcd for ($C_{17}H_{13}BrN_2O$–H) 340.0211; found 340.0217.

4.1.1.30. 6′-Bromo-5-phenyl-1*H*,1′*H*-2,2′-biindole (10g).: Compound **10g** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and *tert*-butyl 2-iodo-5-phenyl-1*H*-indole-1-carboxylate.

Yield = 17%, pale yellow solid. Mp = 237–239 °C. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.78 (s, 1H), 11.69 (s, 1H), 7.85–7.86 (m, 1H), 7.68–7.70 (m, 2H), 7.51–7.56 (m, 2H), 7.42–7.50 (m, 3H), 7.31 (t, J = 7.4 Hz, 1H), 7.14 (dd, J = 8.4, 1.7 Hz, 1H), 7.0 (s, 1H), 6.96 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz): 141.62, 137.79, 136.60, 132.25, 132.00, 131.52, 128.95, 128.76 (2C), 127.46, 126.63 (2C), 126.27, 122.31, 121.74, 121.36, 118.17, 114.19, 113.45, 111.48, 99.30, 98.60. HRMS calcd for $(C_{22}H_{15}BrN_2 - H)^-$ 386.0419; found 386.0429.

4.1.1.31. 5-Bromo-1*H*,1′*H*-2,2′-biindole (10h).: Compound **10h** was prepared from (5-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9b**) and *tert*-butyl 2-iodo-1*H*-indole-1-carboxylate.

Yield = 50%, white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.77 (s, 1H), 11.61 (s, 1H), 7.77 (d, J = 1.7 Hz, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 8.6 Hz, 1H), 7.22 (dd, J = 8.6, 1.9 Hz, 1H), 7.10–7.15 (m, 1H), 7.0–7.04 (m, 1H), 6.95 (d, J = 1.3 Hz, 1H), 6.92 (d, J = 1.4 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): 137.0, 135.60, 132.91, 130.73, 130.37, 128.30, 124.03, 122.12, 121.96, 120.18, 119.50, 112.92, 111.87, 111.14, 99.02, 97.94. HRMS calcd for ($C_{16}H_{11}BrN_2$ H) 310.0106; found 310.0107.

4.1.1.32. 5,5′-Bibromo-1*H*,1′*H*-2,2′-biindole (10i).: Compound **10i** was prepared from (5-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9b**) and 5-bromo-2-iodo-1***H***-indole (**8b**).

Yield = 65%, brown solid. Mp = 304–306 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.83 (s, 2H), 7.79 (d, J = 1.7 Hz, 2H), 7.36 (d, J = 8.6 Hz, 2H), 7.23 (dd, J = 8.6, 1.9 Hz, 2H), 6.94 (d, J = 1.4 Hz, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz): 135.68, 132.22, 130.22, 124.33, 122.30, 113.03, 111.96, 98.52. HRMS calcd for $(C_{16}H_{11}BrN_2 - H)^-$ 387.9211; found 387.9221.

4.1.1.33. 5,6-Dibromo-1*H*,1′*H*-2,2′-biindole (10j).: Compound **10j** was prepared from (1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9c**) and 5,6-dibromo-2-iodo-1*H*-indole (**8e**).

Yield = 53%, white solid. Mp = 233 °C –(decomposition). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.86 (s, 1H), 11.66 (s, 1H), 8.00 (s, 1H), 7.74 (s, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.14 (t, J = 7.2 Hz, 1H), 7.02 (t, J = 7.4 Hz, 1H), 6.97 (s, 1H), 6.94 (s,1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 137.0, 136.7, 133.8, 130.2, 129.6, 128.2, 124.1, 122.1, 120.3, 119.5, 115.4, 115.3, 113.5, 111.2, 99.4, 97.8. HRMS calcd for $(C_{16}H_{10}Br_2N_2 - H)^-$ 388.9118; found 388.9108.

4.1.1.34. 5,6,6′-Tribromo-1*H*,1′*H*-2,2′-biindole (10k).: Compound **10k** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 5,6-dibromo-2-iodo-1*H*-indole (**8e**).

Yield = 24%, pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 11.95 (s, 1H), 11.87 (s, 1H), 8.02 (s, 1H), 7.75 (s, 1H), 7.75 (s, 1H), 7.56 (s, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.15 (dd, J = 1.7 Hz, 8.4 Hz, 1H), 6.98 (br s, 1H), 6.94 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 137.9, 136.7, 133.2, 131.3, 129.5, 127.3, 124.3, 122.5, 122.0, 115.6, 115.5, 114.6, 113.64, 113.60, 99.5, 98.3. HRMS calcd for (C_{16}-$H_9Br_3N_2$–H)$^-$ 466.8223; found 466.8223.

4.1.1.35. 5,5′,6,6′-Tetrabromo-1*H*,1′*H*-2,2′-biindole (10l).: Compound **10l** was isolated as a minor bi-product during the synthesis of **10k** from **9a** and **8e**.

Yield = 7%, pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.98 (bd, J = 0.8 Hz, 2H), 8.04 (s, 2H), 7.75 (d, J = 0.6 Hz, 2H), 6.97 (d, J = 1.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 136.8 (2C), 132.6 (2C), 129.3 (2C), 124.5 (2C), 115.9 (2C), 115.6 (2C), 113.8 (2C), 98.9 (2C). HRMS calcd for ($C_{16}H_8Br_4N_2$–H)$^-$ 546.7308; found 546.7292.

4.1.1.36. 5,5′,6-Tribromo-1*H*,1′*H*-2,2′-biindole (10m).: Compound **10m** was prepared from (5-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9b**) and 5,6-dibromo-2-iodo-1*H*-indole (**8e**).

Yield = 49%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.04 (s, 1H), 11.97 (s, 1H), 8.02 (s, 1H), 7.79 (d, J = 1.8 Hz, 1H), 7.75 (s,1H), 7.37 (d, J = 8.6 Hz, 1H), 7.23 (dd, J = 1.9 Hz, 8.6 Hz, 1H), 6.95 (br s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 136.8, 135.7, 133.2, 131.8, 130.1, 129.5, 124.5, 124.3, 122.4, 115.58, 115.55, 113.6, 113.1, 112.0, 98.9, 98.4. HRMS calcd for ($C_{16}H_9Br_3N_2$–H)$^-$ 466.8223; found 466.8211.

4.1.1.37. 5,6′-Dibromo-1-methyl-1*H*,1′*H*-2,2′-biindole (12a).: Compound **12a** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 5-bromo-2-iodo-1-methyl-1*H*-indole (**11b**).

Yield = 53%, white solid. Mp = 180–182 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.78 (s, 1H), 7.82 (d, J = 1.9 Hz, 1H), 7.51–7.61 (m, 3H), 7.31 (dd, J = 8.7, 2.0 Hz, 1H), 7.18 (dd, J = 8.4, 1.8 Hz, 1H), 6.91 (s, 1H), 6.89 (s, 1H), 3.96 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): 137.58, 137.0, 133.90, 130.03, 128.85, 127.41, 124.25, 122.45, 122.31, 122.06, 114.75, 113.68, 112.35, 112.14, 101.76, 100.61, 31.78. HRMS calcd for ($C_{17}H_{12}Br_2N_2$–H)$^-$ 401.9367; found 401.9384.

4.1.1.38. 5′,6-Dibromo-1-methyl-1*H*,2′*H*-2,2′-biindole (12b).: Compound **12b** was prepared from (5-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9b**) and 6-bromo-2-iodo-1-methyl-1*H*-indole (**8a**).

Yield = 48%, pale yellow solid. Mp = 192–194 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.76 (s, 1H), 7.84 (s, 1H), 7.78 (d, J = 1.9 Hz, 1H), 7.57 (d, J = 8.4, 1H), 7.39 (d, J = 8.6 Hz, 1H), 7.26 (dd, J = 8.6, 1.9 Hz, 1H), 7.21 (dd, J = 8.4, 1.7 Hz, 1H), 6.92 (d, J = 0.6 Hz, 1H), 6.86 (d, J = 1.5 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): 139.13, 135.39, 133.41, 130.59, 130.25, 126.04, 124.57, 122.71, 122.37, 121.92, 114.76, 113.16, 112.97, 111.92, 101.48, 101.06, 31.78. HRMS calcd for (C17H12Br2N2--H)$^-$ 401.9367; found 401.9376.

4.1.1.39. 5,6′-Dibromo-1-(methoxymethyl)-1*H*,1′*H*-2,2′-biindole (12c).: Compound **12c** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 5-bromo-2-iodo-1-(methoxymethyl)-1*H*-indole (**11c**).

Yield = 65%, white solid. Mp = 199–201 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.74 (s, 1H), 7.87 (d, J = 1.9 Hz, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.55–7.61 (m, 2H), 7.36 (dd, J = 8.3, 2.4 Hz, 1H), 7.17 (dd, J = 8.4, 1.8 Hz, 1H), 6.98 (s, 1H), 6.95 (s, 1H), 5.71(s, 2H) 3.30 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): 137.69, 137.46, 133.97, 129.50, 129.18, 127.40, 124.95, 122.55, 122.45, 122.26, 114.87, 113.67, 113.14, 112.51, 102.23, 101.93, 74.19, 55.55. HRMS calcd for $(C_{18}H_{14}Br_2N_2O–H)^-$ 431.9473; found 431.9485.

4.1.1.40. 2-(5,6′-Dibromo-1*H*,1′*H*-[2,2′-biindol]-1-yl)acetic acid (14).: Compound **14** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 2-(5-bromo-2-iodo-1*H*-indol-1yl)acetic acid (**13**).

Yield = 33%, white solid. Mp = 321–323 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.77 (s, 1H), 7.84 (d, J = 1.9 Hz, 1H), 7.52–7.60 (m, 3H), 7.31 (dd, J = 8.7, 2.0 Hz, 1H), 7.17 (dd, J = 8.5, 1.7 Hz, 1H), 6.91 (s, 1H), 6.69 (d, J = 1.5 Hz, 1H), 5.24 (s, 2H). ^{13}C NMR (DMSO-d_6, 150 MHz): 170.07, 137.48, 137.29, 133.85, 129.69, 129.03, 127.34, 124.57, 122.51, 122.39, 122.13, 114.84, 113.67, 112.71, 112.34, 101.64, 100.79, 46.05. HRMS calcd for $(C_{18}H_{12}Br_2N_2O_2–H)^-$ 445.9266; found 445.9272.

4.1.1.41. 2-(5,6′-Dibromo-1*H*,1′*H*-[2,2′-biindol]-1-yl)ethanol (17).: Compound **17** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 2-(5-bromo-2-iodo-1*H*-indol-1-yl)ethanol (**16**).

Yield = 8%, pale white solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.71 (s, 1H), 7.81 (d, J = 1.9 Hz, 1H), 7.54–7.59 (m, 3H), 7.30 (dd, J = 8.7, 2.0 Hz, 1H), 7.18 (dd, J = 8.4, 1.8 Hz, 1H), 6.91 (s, 1H), 6.86 (s, 1H), 5.17 (t, J = 5.1 Hz, 1H), 4.47 (t, J = 5.9 Hz, 2H), 3.78 (q, J = 5.6 Hz, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz): 137.48, 136.82, 133.84, 130, 129.06, 127.36, 124.22, 122.42, 122.26, 122.07, 114.66, 113.70, 112.74, 112.39, 101.74, 101.32, 59.97, 46.44. HRMS calcd for $(C_{18}H_{14}Br_2N_2O–H)^-$ 431.9473; found 431.9483.

4.1.1.42. 5,6′-Dibromo-1-(2-(4-methylpiperazin-1-yl)ethyl)-1*H*,1′*H*-2,2′-biindole (20a).: Compound **20a** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 5-bromo-2-iodo-1-(2-(4-methylpiperazin-1-yl)ethyl)-1*H*-indole (**19a**).

Yield = 57%, pale white solid. Mp: 140 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 11.94 (s, 1H), 7.81 (s, 1H), 7.52–7.61 (m, 3H), 7.31 (d, J = 8.7 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.89 (s, 1H), 6.84 (s, 1H), 4.51 (t, J = 6.4 Hz, 2H), 2.62 (t, J = 6.4 Hz, 2H), 2.15–2.45 (m, 8H), 2.11 (s, 3H). ^{13}C NMR (150 MHz): 137.47, 136.46, 133.66, 130.04, 129.12, 127.38, 124.34, 122.45, 122.41, 122.10, 114.65, 113.72, 112.54, 112.49, 101.71, 101.50, 56.77, 54.39, 52.79, 45.38, 42.41. HRM Scalcd for ($C_{23}H_{24}Br_2N_4$ H) 514.0368; found 514.0372.

4.1.1.43. 4-(2-(5,6′-Dibromo-1*H*,1′*H*-[2,2′-biindol]-1-yl)ethyl)morpholine (20b).: Compound **20b** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 4-(2-(5-bromo-2-iodo-1*H*-indol-1-yl)ethyl)morpholine (**19b**).

Yield = 22%, white form. 1H NMR (400 MHz, DMSO-d6): δ 11.89 (s, 1H), 7.81 (d, J = 1.3 Hz, 1H), 7.59–7.56 (m, 3H), 7.31 (dd, J = 1.4 Hz, 8.7 Hz, 1H), 7.18 (dd, J = 1.2 Hz, 8.4 Hz, 1H), 6.89 (s, 1H), 6.85 (s, 1H), 4.54 (t, J = 6.4 Hz, 2H), 3.43 (br s, 4H), 2.60 (t, J = 6.3 Hz, 2H), 2.30 (br s, 4H). 13C NMR (100 MHz, DMSO-d_6): δ 137.5, 136.5, 133.7, 130.0, 129.1, 127.3, 124.3, 122.4, 122.3, 122.1, 114.6, 113.7, 122.5, 122.4, 101.7, 101.4, 66.0, 57.3, 53.5, 1720 N. S. Kumar et al. / Bioorg. Med. Chem. 22 (2014) 1708–1725 42.0. HRMS calcd for $(C_{22}H_{21}Br_2ON_3–H)^-$ 504.0105, found 504.0116.

4.1.1.44. 2-(6-Bromo-1*H*-indol-2-yl)benzo[*d*]thiazole (22a).: Compound **22a** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 2-iodobenzo[d]thiazole (**21a**).

Yield = 43%, pink solid. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 12.35 (s, 1H), 8.16 (ddd, J = 8.0, 1.1, 0.6 Hz, 1H), 8.04 (ddd, J = 8.2, 1.1, 0.6 Hz, 1H), 7.63–7.64 (m, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.57 (ddd, J = 8.2, 7.2, 1.2 Hz, 1H), 7.48 (ddd, J = 8.2, 7.2, 1.2 Hz, 1H), 7.29 (dd, J = 2.2, 0.8 Hz, 1H), 7.21 (dd, J = 8.4, 1.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz): 159.40, 153.21, 150.64, 138.43, 134.23, 131.92, 126.85, 126.78, 125.60, 123.21, 123.10, 122.50, 122.41, 116.61, 114.63, 105.08. HRMS calcd for $(C_{15}H_9BrN_2S–H)^-$ 327.9670; found 327.9674.

4.1.1.45. 2-(Benzofuran-2-yl)-6-bromo-1*H*-indole (22b).: Compound **22b** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 2-iodobenzofuran (**21b**).

Yield = 40%, pale white solid. Mp = 196–198 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.02 (s, 1H), 7.71 (d, J = 7.1 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.60 (s, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.25–7.37 (m, 3H), 7.18 (dd, J = 8.4, 1.7 Hz, 1H), 7.0 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): 154.0, 149.12, 137.97, 129.34, 128.55, 127.11, 124.75, 123.44, 122.75, 122.26, 121.55, 115.11, 113.92, 111.0, 102.28, 100.45. HRMS calcd for $(C_{16}H_{10}BrNO–H)^-$ is 310.9946; found 310.9948.

4.1.1.46. 2-(Benzo[*b*]thiophen-2-yl)-6-bromo-1*H*-indole (22c).: Compound **22c** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and 2-iodobenzo[*b*]thiophene (**21c**).

Yield = 57%, white solid. Mp = 250–252 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.91 (s, 1H), 7.94–7.98 (m, 1H), 7.86 (dd, J = 7.1, 1.5 Hz, 1H), 7.82 (s, 1H), 7.54–7.57 (m, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.33–7.43 (m, 2H), 7.14 (dd, J = 8.4, 1.8 Hz, 1H), 6.83 (d, J = 1.4 Hz). ^{13}C NMR (DMSO-d_6, 150 MHz): 139.98, 138.35, 138.03, 135.14, 132.93, 127.38, 124.99, 124.86, 123.75, 122.67, 122.47, 122.01, 119.87, 114.87, 113.67, 100.86. HRMS calcd for $(C_{16}H_{10}BrNS–H)^-$ 326.9717, found 326.9708.

4.1.1.47. 2-(5-Bromo-1*H*-indol-2-yl)benzo[*d*]thiazole (22d).: Compound **22d** was prepared from (5-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9b**) and 2-iodobenzo[*d*]thiazole (**21a**).

Yield = 35%, colorless solid. Mp = 208–210 °C. ^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 12.43 (s, 1H), 8.15 (d, J = 7.9 Hz, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.84 (d, J = 1.5 Hz, 1H), 7.57 (t, J = 7.7 Hz, 1H), 7.42–7.50 (m, 2H), 7.34 (dd, J = 8.7, 1.8 Hz, 1H), 7.22 (s, 1H). ^{13}CNMR (DMSO-d_6, 100 MHz): 159.35, 153.22, 136.32, 134.22, 132.31, 129.50, 126.80, 126.44, 125.58, 123.30, 122.44 (2C), 114.22, 112.59, 104.24. HRMS calcd for ($C_{15}H_9BrN_2S$–H)$^-$ 327.9670; found 327.9676.

4.1.1.48. 2-(Benzofuran-2-yl)-5-bromo-1*H*-indole (22e).: Compound **22e** was prepared from (5-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9b**) and 2-iodobenzofuran (**21b**).

Yield = 38%, white solid. Mp = 236–238 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.06 (s, 1H), 7.80 (d, J = 1.9 Hz, 1H), 7.70–7.73 (m, 1H), 7.61–7.66 (m, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.25–7.38 (m,4H), 6.97 (d, J = 1.5 Hz, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz): 154.09, 154.08, 149.06, 149.04, 135.82, 129.91, 129.81, 128.50, 124.98, 124.80, 123.44, 122.64, 121.29, 113.42, 112.20, 111.01, 102.41, 99.81. HRMS calcd for $C_{16}H_{10}BrNO$ is 310.9946; found 310.9954.

4.1.1.49. 2-(Benzo[*b*]thiophen-2-yl)-5-bromo-1*H*-indole (22f).: Compound **22f** was prepared from (5-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9b**) and 2-iodobenzo[*b*]thiophene (**21c**).

Yield = 57%, white solid. Mp = 269–271 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.0 (s, 1H), 7.98–8.01 (m, 1H), 7.86–7.90 (m, 1H), 7.85 (s, 1H), 7.76 (d, J = 1.8 Hz, 1H), 7.35–7.44 (m, 3H), 7.25 (dd, J = 8.6, 1.9 Hz, 1H), 6.82 (d, J = 1.6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): 139.97, 138.37, 135.90, 134.64, 133.38, 130.18, 124.97, 124.89, 124.83, 123.75, 122.48, 122.38, 120.03, 113.24, 112.15, 100.26. HRMS calcd for ($C_{16}H_{10}BrNS$–H)$^-$ 326.9717; found 326.9722.

4.1.1.50. 6-Bromo-2-(5-bromo-1*H*-indol-2-yl)benzo[*d*]thiazole (22g).: Compound **22g** was prepared from (5-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9b**) and 6-bromo-2-iodobenzo[*d*]thiazole (**21c**).

Yield = 48%, yellow solid. Mp = 246–248 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.45 (s, 1H), 8.48 (d, J = 2.0 Hz, 1H), 7.97 (d, J = 8.7 Hz, 1H), 7.87 (d, J = 1.8 Hz, 1H), 7.72 (dd, J = 8.7, 2.0 Hz, 1H), 7.44 (d, J = 8.7 Hz, 1H), 7.36 (dd, J = 8.7, 1.9 Hz, 1H), 7.28 (d, J = 1.5 Hz, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz): 160.26, 152.30, 136.42, 136.23, 131.87, 129.93, 129.45, 126.64, 125.01, 123.85, 123.38, 118.00, 114.26, 112.66, 104.67. HRMS calcd for ($C_{15}H_8Br_2N_2S$–H)$^-$ 405.8775; found 405.8765.

4.1.1.51. 2-(4-Bromo-3-nitrophenyl)-1*H*-indole (34a).: Compound **34a** was prepared from (1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid and 1,4-dibromo-2-nitrobenzene.

Yield = 38%, brown form. 1H NMR (400 MHz, CDCl3): δ 8.45 (br s,1H), 7.96 (d, J = 2.0 Hz, 1H), 7.76 (dd, J = 2.0 Hz, 8.4 Hz, 1H), 7.65 (dd, J = 0.9 Hz, 7.9 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H) 7.42 (dd, J = 0.8 Hz, 8.2 Hz, 1H), 7.28–7.24 (m, 1H), 7.17–7.13 (m, 1H), 6.73–6.72 (m, 1H).

4.1.1.52. 2-(4-Bromo-3-methoxyphenyl)-1*H*-indole (34b).: Compound **34b** was prepared from (1-(*tert*-butox-ycarbonyl)-1*H*-indol-2-yl)boronic acid and 1,4-dibromo-2-methoxybenzene.

Yield = 57%, white solid. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.59 (s, 1H), 7.63 (d, J = 8.2 Hz, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.42–7.39 (m, 2H), 7.14–7.11 (m, 1H), 7.02–7.00 (m, 2H). ^{13}C NMR (DMSO-d_6, 150 MHz): 156.2, 137.6, 137.2, 133.7, 133.6, 129.0, 122.4, 120.7, 120.0, 119.0, 111.8, 109.8, 109.5, 100.1, 56.9.

4.1.1.53. 1,4-Bis-(6-bromo-1*H*-indol-2-yl)benzene (33a).: Compound 33a was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) (2 equiv) and 1,4-diiodobenzene.

Yield = 67%, white solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.75 (s, 2H), 7.96 (s, 4H), 7.55 (s, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.14 (dd, J = 1.7 Hz, 8.4 Hz, 2H), 7.02 (d, J = 1.5 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 138.2 (2C), 138.0 (2C), 130.8 (2C), 127.7 (2C), 125.5 (4C), 122.3 (2C), 121.8 (2C), 114.2 (2C), 113.6 (2C), 99.2 (2C). HRMS calcd for $(C_{22}H_{14}Br_2N_2–H)^-$ 464.9432; found 464.9441.

4.1.1.54. 2,5-Bis-(6-bromo-1*H*-indol-2-yl)thiophene (33b).: Compound **33b** was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) (2 equiv) and 2,5-diiodothiophene.

Yield = 88%, yellow solid. Decomposes >228 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.81 (s, 2H), 7.55 (s, 2H), 7.53 (br s, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.15 (dd, J = 1.7 Hz, 8.4 Hz, 2H), 6.76 (d, J = 1.2 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 137.8 (2C), 133.8 (2C), 132.8 (2C), 127.5 (2C), 124.9 (2C), 122.6 (2C), 121.7 (2C), 114.4 (2C), 113.5 (2C), 99.3 (2C). HRMS calcd for $(C_{20}H_{12}Br_2N_2.S–H)^-$ 470.8995, found 470.9004.

4.1.1.55. 2-(4-(1*H*-Indol-2-yl)phenyl)-6-bromo-1*H*-indole (33c).: Compound *33c* was prepared from (6-bromo-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**9a**) and *tert*-butyl 2-(4-iodophenyl)-1*H*-indole-1-carboxylate.

Yield = 89%, Yellow solid. Mp P300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.74 (s, 1H), 11.57 (s, 1H), 7.97 (d, J = 8.7 Hz, 1H), 7.94 (d, J = 9.1 Hz, 1H), 7.55 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.15–7.09 (m, 2H), 7.03–6.98 (m, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 138.3, 138.0, 137.2, 137.1, 131.3, 130.4, 128.6, 127.7, 125.5, 125.3 (2C), 125.31 (2C), 122.3, 121.7, 120.0, 119.4, 114.1, 113.6, 111.2, 99.1, 99.0. HRMS calcd for $(C_{22}H_{15}BrN_2–H)^-$ 387.0328; found 387.0333.

4.1.1.56. 2-(4-(1*H*-Indol-2-yl)-3-nitrophenyl)-6-bromo-1*H*-indole (33d).: Compound **33d** was prepared from **9a** and **34a**.

Yield = 57%, brown solid. (Mixture of isomers ~5:1) ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.97 (d, J = 1.3 Hz, 1H), 11.60 (d, J = 1.3 Hz, 1H), 8.45 (d, J = 1.8 Hz, 1H), 8.25 (dd, J = 1.8 Hz, 8.2 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 7.60–7.55 (m, 3H), 7.43 (d, J = 8.1 Hz, 1H), 7.15–7.06 (m, 3H), 7.03 (dd, J = 7.0 Hz, 7.9 Hz, 1H), 6.59 (d,J = 1.4 Hz, 1H). 13C NMR (150 MHz, DMSO-d_6): δ 148.9, 138.3, 137.1, 135.9, 132.1, 131.9, 131.4, 128.3, 128.1, 127.4, 124.4, 122.8, 122.4, 122.3, 120.5, 119.9, 119.6, 115.2, 113.9, 111.5, 101.7, 101.2. HRMS calcd for $(C_{22}H_{14}BrN_3O_2–H)^-$ 432.0179; found 432.0195.

4.1.1.57. 2-(4-(1*H*-Indol-2-yl)-2-methoxyphenyl)-6-bromo-1*H*-indole (33e).: Compound **33e** was prepared from **9a** and **34b**.

Yield = 11%, yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.62 (d, J = 1.0 Hz, 1H), 11.37 (d, J = 1.0 Hz, 1H), 7.87 (d, J = 8.1 Hz, 1H), 7.65 (d, J = 1.3 Hz, 1H), 7.63 (s, 1H), 7.59 (dd, J = 1.6 Hz, 8.1 Hz, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.14–7.11 (m, 2H), 7.06–7.01 (m, 3H), 4.09 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 156.4, 137.3, 137.2, 137.2, 135.4, 132.6, 128.6, 128.0, 127.2, 122.0, 121.8, 121.5, 120.1, 119.5, 118.9, 117.6, 113.8, 113.7, 111.2, 108.4, 101.7, 99.5, 55.9. HRMS calcd for $(C_{23}H_{17}BrN_2O–H)^-$ 417.0434; found 417.0442.

4.1.1.58. 2-(3-(1*H*-Indol-2-yl)phenyl)-6-bromo-1*H*-indole (33f).: Compound **33f** was prepared from **9a** and *tert*-butyl 2-(3-iodo-phenyl)-1*H*-indole-1-carboxylate.

Yield = 86%, white solid. Mp = 224–226 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.78 (s, 1H), 11.60 (s, 1H), 8.38 (s, 1H), 7.82 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.58–7.54 (m, 4H), 7.44 (d, J = 7.4 Hz, 1H), 7.17–7.01 (m, 5H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 138.5, 138.0, 137.3, 137.1, 132.9, 132.3, 129.5, 128.6, 127.6, 124.2, 123.9, 122.3, 121.9, 121.8, 121.7, 120.1, 119.4, 114.2, 113.7, 111.3, 99.3, 99.2. HRMS calcd for $(C_{22}H_{15}BrN_2–H)^-$ 387.0328; found 387.0335.

4.1.1.59. Specific procedure for the synthesis of 2-(5,6′-dibromo-1*H*,1′*H*-[2,2′-biindol]-1-yl)-1-morpholinoethanone (15).: To a stirred solution of compound **14** (50 mg, 0.11 mmol), morpholine (10 μL, 0.12 mmol) and HBTU (44 mg, 0.12 mmol) in DMF (1 ml) at 0 °C was added DIPEA (61 μL, 0.35 mmol) and the resulting solution stirred at rt for 12 h. The mixture was diluted with DCM (10 ml) and washed with 1 N HCl, saturated aqueous $NaHCO_3$, brine and concen-trated. The crude product was purified by automated flash chroma-tography to give compound 15 as white solid (38 mg, 68%).

Mp = 320–322 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.17 (s, 1H), 7.65 (s, 1H), 7.50 (s, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 8.7 Hz, 1H), 7.11 (d, J = 8.5 Hz, 1H), 7.07 (d, J = 8.5 Hz, 1H), 6.71 (s, 1H), 6.37 (s, 1H), 5.10 (s, 2H), 3.62 (br s, 2H), 3.54 (br s, 2H). HRMS calcd for $(C_{22}H_{19}Br_2N_3O_2–H)^-$ 514.9844; found 514.9867.

4.1.1.60. General procedure for the synthesis of substituted 2-ethynyl-1*H*-indole (24) and substituted bis-indole (25).: A solution of 2-iodoindole **7** (1 mmol) and acetylene derivative (1 mmol) in THF (5 ml) was purged with argon for 10 min followed by triethylamine (3.5 mmol), CuI (0.2 mmol) and $Pd(Ph_3P)_2Cl_2$ as catalyst (10 mol %). The

mixture was heated in lwave at 100 °C for 30 min. After completion of the reaction as monitored by TLC, water was added and the mixture extracted with EtOAc (2 20 ml). Combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified by automated flash chromatography and then taken up in THF (5 ml) followed by the addition if TBAF (1 M in THF, 1 mmol). The mixture was stirred at rt for 2 h and then partitioned between EtOAc (50 ml) and H_2O (50 ml). The organic phase was washed with brine (50 ml), dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by automated flash chromatogra-phy using EtOAc and hexanes as eluents to give the desired product.

4.1.1.61. 6-Bromo-2-ethynyl-1*H*-indole (24a).: Compound **24a** was prepared from **7a** and TIPS-acetylene.

Yield = 52%, dark brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20 (s, 1H), 7.48 (s, 1H), 7.44 (d, J = 8.3, 1.0 Hz, 2H), 7.23 (dd, J = 8.5, 1.6 MHz, 1H), 6.68 (s, 1H), 3.33 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): 136.59, 126.23, 123.98, 122.17, 118.29, 117.47, 113.54, 109.61, 81.31, 75.55. HRMS calcd for $(C_{10}H_6Br_2N–H)^-$ 218.9684; found 218.9687.

4.1.1.62. 5-Bromo-2-ethynyl-1*H*-indole (24b).: Compound **24b** was prepared from **7b** and TIPS-acetylene.

Yield = 63%, brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.23 (s, 1H), 7.72 (d, J = 1.32 Hz, 1H), 7.32 (dd, J = 8.7, 1.8 Hz, 1H), 7.19 (d, J = 8.7 Hz, 1H), 6.75 (d, J = 1.5 Hz, 1H), 3.33 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): 134.59, 129.20, 126.82, 123.52, 118.93, 113.96, 112.38, 109.15, 81.54, 75.77. HRMS calcd for $(C_{10}H_6BrN–H)^-$ 218.9684; found 218.9685.

4.1.1.63. 2-((1*H*-Indol-2-yl)ethynyl)-6-bromo-1*H*-indole (25a).: Compound **25a** was prepared from **24a** and 2-iodo-1-(phenylsulfo-nyl)-1*H*-indole.

Yield = 64%, pale brown solid; Mp = 240–242 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.94 (s, 1H), 11.78 (s, 1H), 7.57 (dd, J = 7.9, 0.6 Hz, 1H), 7.52–7.54 (m, 2H), 7.36 (dd, J = 8.2, 0.9 Hz, 1H), 7.17–7.22 (m, 2H), 7.06 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 6.89 (dd, J = 2.0, 0.7 Hz, 1H), 6.88 (dd, J = 2.0, 0.9 Hz, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz): δ 150.63, 137.31, 136.61, 127.15, 126.22, 123.23, 122.91, 122.22, 120.50, 119.97, 118.80, 117.50, 115.92, 113.78, 113.39, 108.33, 108.16, 85.62, 84.50. HRMS calcd for $(C_{18}H_{11}BrN_2–H)^-$ 334.0106; found 334.0113.

4.1.1.64. 1,2-Bis-(6-bromo-1*H*-indol-2-yl)ethyne (25b).: Compound **25b** was prepared from coupling **7a** with **24a**.

Yield = 50%, yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.24 (br s, 2H), 7.52 (s, 2H), 7.47 (d, J = 8.2 Hz, 2H), 7.26 (m, 2H), 6.84 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 137.0 (2C), 126.5 (2C), 124.3 (2C), 122.2 (2C), 118.6 (2C), 117.7 (2C), 113.8 (2C), 109.7 (2C), 85.1 (2C). HRMS calcd for $(C_{18}H_{10}Br_2N_2–H)^-$ 412.9118; found 412.9126.

4.1.1.65. 6-Bromo-2-((5-bromo-1*H*-indol-2-yl)ethynyl)-1*H*-indole (25c).: Compound **25c** was prepared from **7b** and **24a**. Yield = 32%, pale yellow solid; Mp = 236–237 °C; ^{1}H NMR

($CDCl_3$, 600 MHz): δ 12.03 (s, 1H), 11.96 (s, 1H), 7.77 (d, J = 1.8 Hz, 1H), 7.52–7.55 (m, 2H), 7.33 (d, J = 8.7 Hz, 1H), 7.30 (dd, J = 8.6, 1.9 Hz, 1H), 7.19 (dd, J = 8.5, 1.6 Hz, 1H), 6.93 (m, 1H), 6.86 (m, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz): 137.78, 135.60, 129.35, 126.61, 126.12, 123.37, 122.97, 122.72, 119.45, 118.88, 116.50, 114.26, 113.75, 112.90, 108.93, 108.14, 85.50, 85.38. HRMS calcd for $(C_{18}H_{10}Br_2N_2–H)^-$ 412.9118; found 412.9102.

4.1.1.66. Synthesis of compounds 26a and 26b.: A mixture of **25b** (70 mg, 0.17 mmol), K_2CO_3 (94 mg, 0.65 mmol) and methyl io-dide (19 μL, 0.30 mmol) in DMF (1.5 ml) was stirred at 30 °C for 3δ and then partitioned between EtOAc (20 ml) and H_2O (10 ml). The organic phase was washed with brine (10 ml), dried over anhy-drous Na_2SO_4 and concentrated. The crude product was purified by automated flash chromatography using EtOAc and hexanes as eluents to give compounds **26a** and **26b**.

4.1.1.67. 6-Bromo-2-((6-bromo-1*H*-indol-2-yl)ethynyl)-1-methyl-1*H*-indole (26a).: Yield = 40%, yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.99 (s, 1H), 7.79 (s, 2H), 7.56 (s, 1H), 7.54 (d, J = 8.4 Hz, 2H), 7.23 (dd, J = 1.6 Hz, 8.4 Hz, 1H), 7.19 (dd, J = 1.7 Hz, 8.5 Hz, 1H), 6.96 (s, 2H), 3.89 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 138.0, 137.4, 126.2, 125.6, 123.2, 123.0, 122.4, 122.3, 121.7, 118.4, 116.4, 116.1, 113.8, 113.1, 108.5, 107.6, 88.4, 83.6, 30.9. HRMS calcd for $(C_{19}H_{12}Br_2N_2–H)^-$ 426.9275; found 426.9279.

4.1.1.68. 1,2-Bis-(6-bromo-1-methyl-1*H*-indol-2-yl) ethyne (26b).: Yield = 47%, pale brown solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.82 (s, 2H), 7.55 (d, J = 8.3 Hz, 2H), 7.24 (d, J = 8.3 Hz, 2H), 7.03 (s, 2H), 3.90 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 138.0 (2C), 125.6 (2C), 123.2 (2C), 122.5 (2C), 121.5 (2C), 116.5 (2C), 113.1 (2C), 108.1 (2C), 86.9 (2C), 31.0 (2C). HRMS calcd for $(C_{20}H_{14}Br_2N_2+H)^+$ 442.9577; found 442.9562.

4.1.1.69. Synthesis of (E)-2-(2-(1*H*-indol-2-yl)vinyl)-6-bromo-1*H*-indole (27a).: To a stirred solution of **25b** (72 mg, 0.17 mmol) and Et_3N (0.34 ml, 2.4 mmol) in THF (2 ml) at 50 °C under Ar was added formic acid (88%, 77 μL, 1.8 mmol) followed by addition of 10% Pd/C (8 mg). More 10% Pd/C (8 mg) was added every 15 min to a total of 40 mg and the mixture was heated for an additional 2 h, filtered through a pad of silica which was thoroughly washed with EtOAc (2×10 ml). The filtrate was washed with H_2O (10 ml), brine (10 ml), dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by automated flash chromatography using EtOAc and hexanes as eluents to give compounds **27a** as yellow solid (4 mg, 7%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.57 (s, 1H), 11.42 (s, 1H), 7.50 (d, J = 7.9 Hz, 1H), 7.49 (br s, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 7.20 (d, J = 16.5 Hz, 1H), 7.18 (d, J = 16.5 Hz, 1H), 7.11–7.09 (m, 2H), 6.96 (dd, J = 7.1 Hz, 7.8 Hz), 6.57 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 138.2, 137.7, 137.5, 136.5, 128.4, 127.6, 122.18, 122.17, 121.6, 120.0, 119.3, 119.1, 117.7, 114.5, 113.3, 110.9, 103.2, 102.6. HRMS calcd for $(C_{18}H_{13}BrN_2–H)^-$ 335.0189, found 335.0195.

4.1.1.70. Synthesis of compounds 27b and 28a.: To a stirred solution of 6-bromo-1H-indole-2-carbaldehyde (80 mg, 0.36 mmol) and $TiCl_4$ (58 ll, 0.53 mmol) in THF (5 ml) under Ar was added Zn dust (70 mg, 1.1 mmol) gradually over 15 min and the resulting mixture was refluxed for 3 h. After cooling to rt 10% aqueous solution of K_2CO_3 (1 ml) was added and the reaction mix-ture was stirred at rt for 16 h. The mixture was partitioned

between EtOAc (50 ml) and H_2O (20 ml). The organic phase was washed with brine (20 ml), dried over anhydrous Na_2SO_4 and con-centrated. The crude product was purified by automated flash chromatography using EtOAc and hexanes as eluents to give com-pounds **27b** and **28a**.

4.1.1.71. (E)-1,2-Bis-(6-bromo-1*H*-indol-2-yl)ethane (27b).: Yield = 20%, yellow solid. Decomposes >300 LC. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.59 (s, 2H), 7.49 (s, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.20 (s, 2H), 7.11 (dd, J = 1.7 Hz, 8.4 Hz, 2H), 6.60 (d, J = 0.9 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 138.2 (2C), 137.4 (2C), 127.5 (2C), 122.2 (2C), 121.7 (2C), 118.5 (2C), 114.6 (2C), 113.3 (2C), 103.0 (2C). HRMS calcd for ($C_{18}H_{12}Br_2N_2$ H) 414.9275; found 414.9280.

4.1.1.72. 1,2-Bis-(6-bromo-1*H*-indol-2-yl)ethane (28a).: Yield = 53%, yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.18 (s, 2H), 7.46 (s, 2H), 7.46 (d, J = 8.3 Hz, 2H), 7.04 (d, J = 8.3 Hz, 2H), 6.21 (s, 2H), 3.11 (s, 4H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 140.4 (2C), 136.8 (2C), 127.3 (2C), 121.4 (2C), 120.8 (2C), 113.1 (2C), 112.7 (2C), 98.7 (2C), 27.0 (2C). HRMS calcd for $(C_{18}H_{14}Br_2N_2–H)^-$416.9431; found 416.9435.

4.1.1.73. Synthesis of (6-bromo-1-(phenylsulfonyl)-1*H*-indol-2-yl)methanol (29).: 6-Bromo-*N*-benzenesulfonate (1.79 g, 5.3 mmol) in THF (20 ml) was cooled to –78 °C and LDA 1.8 M in THF (4.46 ml, 8.0 mmol) was added. After 30 min at –78 °C and 30 min at rt the mixture was cooled to –78 °C and paraformalde-hyde (206 mg, 6.89 mmol) was added all at once. The reaction was warmed to rt and stirred for 12 h and then quenched by addition of saturated NH_4Cl. The mixture was extracted with EtOAc, the organics were washed with brine, dried over Na_2SO_4, concentrated and purified by automated flash chromatography using EtOAc and hexanes as eluents to give compound **29**.

Yield = 72%, white solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.25 (d, J = 0.6 Hz, 1H), 7.83 (dd, J = 1.1, 8.5 Hz, 1H), 7.61–7.56 (m, 1H), 7.47 (dd, J = 5.0, 10.8 Hz, 1H), 7.38–7.33 (m, 1H), 6.62 (s, 1H), 4.88 (s, 1H). ^{13}C NMR ($CDCl_3$, 126 MHz): 140.72, 138.27, 137.70, 134.47, 129.72, 128.01, 127.39, 126.51, 122.44, 118.88, 117.48, 111.12, 58.53.

4.1.1.74. Synthesis of (*E*)-6-bromo-2-(2-(5-bromo-1-(phenylsul-fonyl)-1*H*-indol-2-yl)vinyl-1-(phenulsulfonyl)-1*H*-indole (31).: Compound **29** (1.0 g, 2.54 mmol) was treated in dry ether (15 ml) at 0 °C with PBr_3 (240 µl, 2.4 mmol) added slowly and then the reaction was stirred at rt for 30 min. After addition of aq KBr, the mixture was extracted with ether and the organics were washed with brine, dried over Na_2SO_4, concentrated to provide the crude bromide as a brown solid which was dissolved in DCM (20 ml) and triphenylphosphine (744 mg, 2.84 mmol) was added and the mixture was stirred at rt overnight. The solvent was removed in vacuo and the residue was suspended in EtOAc (15 ml), sonicated and the solid collected by filtration. The crude Wittig reagent (1.0 g, 1.49 mmol) was dissolved in 1:1, THF/MeOH (20 ml) and DBU (285 µl, 2.02 mmol) was added followed by 5-bromo-N-benzenesulfonylindole (490 mg, 1.35 mmol). The mixture was stirred at rt for 3 h and then the solvent was removed under reduced pressure and the residue was partitioned between water and EtOAc, and the aqueous layers were washed with EtOAc, the organic extracts were combined, washed with brine dried over Na_2SO_4, concentrated and

purified by automated flash chromatog-raphy using EtOAc and hexanes as eluents to give compound **31** as a yellow solid.

Yield 36%; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.23 (s, 1H), 8.04 (d, J = 8.9 Hz, 1H), 7.85 (d, J = 1.9 Hz, 1H), 7.83–7.48 (m, 15H), 7.35 (s, 1H), 7.27 (s, 1H). ^{13}C NMR (DMSO-d_6, 151 MHz): 139.40, 138.69, 137.45, 137.14, 136.81, 136.72, 135.60, 135.10, 135.02, 131.62, 130.20, 130.09, 130.03, 129.92, 128.77, 128.05, 127.65, 126.45, 126.24, 126.21, 123.81, 123.25, 121.68, 121.58, 118.16, 117.13, 117.02, 116.50, 110.27, 109.61. HRMS calcd for $(C_{30}H_{20}Br_2N_2O_4S_2–H)^-$ 692.9153; found 692.9158.

4.1.1.75. Synthesis of (*E*)-6-bromo-2-(2-(5-bromo-1*H*-indol-2-yl)vinyl)-1*H*-indole (27c).: Compound **31** (20 mg, 0.29 mmol) was dissolved in 1 ml of THF/MeOH, (2:1) and Cs_2CO_3 (28 mg, 0.86 mmol) was added and the mixture has heated in a lwave reactor at 90 °C for 30 min. The reaction was cooled, solvents were removed and the residue was stirred with H_2O (1 ml) for 10 min and then the mixture was extracted with DCM. The extracts were dried (Na_2SO_4), concentrated to dryness and the residue was purified by automated flash chromatography using EtOAc and hexanes as eluents to give compound **27c** as a yellow solid.

Yield = 88%, yellow solid; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.65 (s, 1H), 11.60 (s, 1H), 7.68 (d, J = 1.6 Hz, 1H), 7.50 (s, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.31 (d, J = 8.5 Hz, 1H), 7.22–7.17 (m, 3H), 7.11 (dd, J = 1.7, 8.4 Hz, 1H), 6.60 (s, 1H), 6.56 (s, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz): 138.27, 137.96, 137.38, 136.11, 130.33, 127.52, 124.44, 122.23, 122.05, 121.72, 118.86, 118.47, 114.69, 113.29, 112.84, 111.74, 103.12, 102.3. HRMS calcd for $(C_{18}H_{12}Br_2N_2–H)^-$ 412.9289; found 412.9251.

4.1.1.76. Synthesis of 6-bromo-2-(2-(5-bromo-1*H*-indol-2-yl)ethyl)-1*H*-indole (28b).: Compound **27c** (48 mg) was dissolved in 1:1 EtOAc/MeOH (2 ml) and 5 mg 10% Pt-C was added and the mixture was stirred under a H_2 atmosphere at rt following the reaction by TLC. When complete, the mixture was filtered, concentrated to dryness and the residue was purified by automated flash chromatography using EtOAc and hexanes as eluents to give compound **28b** as a white solid.

Yield = 81%. Mp = 208–210 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.23 (s, 1H), 11.17 (s, 1H), 7.58 (d, J = 1.7 Hz, 1H), 7.45 (s, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 8.6 Hz, 1H), 7.10 (dd, J = 1.9, 8.5 Hz, 1H), 7.04 (dd, J = 1.8, 8.3 Hz, 1H), 6.20 (s, 1H), 6.18 (s, 1H), 3.14 (s, 4H). ^{13}C NMR (DMSO-d_6, 126 MHz): 141.00, 140.39, 136.83, 134.61, 130.18, 127.27, 122.51, 121.41, 121.32, 120.86, 113.14, 112.69, 112.53, 111.15, 98.68, 98.22, 27.04 (2C). HRMS calcd for $(C_{18}H_{14}Br_2N_2–H)^-$ 414.9445; found 414.9470.

4.2. PK enzymatic assays, cell-based assays and cytotoxicity

4.2.1. Pyruvate kinase enzymes and inhibitory activity testing—The pyruvate kinase enzymes MRSA PK and human PK isoforms were identical to and were sourced as His-tagged constructs as described in Ref. 4. Synthetic candidate MRSA PK inhibitors were diluted in 100% DMSO and assayed for their abilities to inhibit enzymatic activities of MRSA PK and (for selected compounds) human PKs as described in Ref. 4.

4.2.2. In vitro susceptibility testing *Staphylococcus aureus* (ATCC29213)— Determinations of in vitro susceptibility and MIC determinations in *S. aureus* ATCC29213 were carried out as described in Ref. 4 except that serial (twofold) dilutions of test compounds were done in 100% DMSO and added to give a final concentration of DMSO of 5% in brain heart infusion (BHI) broth (Difco).

4.2.3. In vitro susceptibility testing (MRSA)—MIC values for selected antimicrobial actives were determined with MRSA strain MW2 (USA400) using the 96-well microtiter CLSI recommended standard for twofold serial broth microdilution method[13] as described previously.[4,7] Each compound was prepared in DMSO with twofold serial dilutions to give a final concentration of 64–0.031 μg/ml. 10 μl of the compound solution was then added, in duplicate, to either, 190 μl of cation adjusted Mueller Hinton broth (CAMHB) or 190 μl CAMHB containing ~2.5×10^5 CFU/ml of bacteria (final compound concentration 64–0.031 μg/ml). Culture plates were then incubated for 18–24 h at 37 °C and the optical density at 590 nm (OD_{590}) was measured using a Tecan M200 (Tecan). The absorbance control values for the series containing CAMHB and inhibitor were subtracted as background from the corresponding infected wells. The MIC was defined as the lowest concentration of test compound leading to complete inhibition of cell growth in relationship to compound-free control wells as determined by optical density. Vancomycin was used as a reference compound. Experiments were replicated at least three times to verify reproducibility using the above conditions.

4.2.4. The effect of efflux inhibitor verapamil on in vitro susceptibility testing —MIC values for selected antimicrobial actives were determined in the presence of efflux inhibitor verapamil with *S. aureus* (ATCC29213) using the 96-well microtiter procedure described above. A sub-inhibitory concentration of verapamil (200 mg/L)[11] was added to CAMHB and all assays were preformed as described above with either CAMHB or CAMHB containing verapamil (vCAMHB). Culture plates were then incubated for 18–24 h at 37 LC and the optical density at 600 nm (OD_{600}) was measured using the PowerWave™ XS (BioTek). The absorbance control values for the series containing CAMHB or vCAMHB and inhibitor were subtracted as background from the corresponding infected wells. The MIC was defined as the lowest concentration of test compound leading to complete inhibition of cell growth in relationship to compound-free control wells as determined by optical density. Vancomycin was used as a reference compound. All com-pounds with the exception of **10l**, were replicated at least three times to verify reproducibility using the above conditions. Compound **10l** was replicated twice due to a shortage of the compound.

4.2.5. Construction of Pyk-deficient bacterial strains and growth conditions— The *S. aureus* Δ*pyk*::Erm^R LAC mutant (AR0891) was constructed by amplifying ~1 kb 5′ and 3′ homology fragments sur-rounding the *pyk* gene (SACOL1745) with the following primers: pyk-5.1A (tggtagaattcGCAGTTTTAACTAGTGGTGG) pyk-5.1B (tggta-gaattcGCTGGTCCAA-TTGTACATAC), pyk-3.1A (gtaggatccACGATTG ATGCTGCTCAAGG), pyk-3.1B (gtagg-atccAGAAGGTGTTTGATCTTG AGC). The resulting fragments were then cloned into the *Eco*RI and *Bam*HI sites flanking an Erm^R cassette, which was cloned into the *Sma*I site of the *Escherichia coli/S. aureus* shuttle vector

pBT2[14] to yield pNV026. Following transformation of pNV026 into *S. aureus* SF8300, allelic exchange was performed as previously described[12] using TSB without glucose supplemented with 1% sodium pyruvate (P). The *pyk*::Erm^R mutation was then transduced into *S. aureus* LAC using bacteriophage Φ80.

4.2.6. Bacterial killing assay—Wild-type *S. aureus* LAC and *S. aureus* LAC Δ*pyk*::Erm^R (AR0891) were grown overnight in TSB without glucose supplemented with 1% sodium pyruvate with shaking at 37 °C. Overnight cultures were washed 2× with sterile PBS and then diluted to an OD600 = 0.1 (~2.5 × 10^7 cfu/ml) in pre-warmed (37 °C) media (either BHI or TSB without glucose supplemented with 1% sodium pyruvate) ±1.6 μM **10d**. Cultures were then grown at 37 °C with shaking at 250 rpm. Viable bacterial were enumerated by dilution plating at 0, 8, and 24 h post-inoculation using Tryptic Soy Agar (TSA) without glucose supplemented with 1% sodium pyruvate.

4.2.7. Mammalian cytotoxicity—Mammalian cytotoxicity was performed using procedures and statistical analysis previously described.[4]

Acknowledgments

The authors thank Dr. Jianghong An of the Genome Sciences Centre of Vancouver for valuable assistance in preparing Figure 2. The authors acknowledge Genome British Columbia, the Natural Sciences and Engineering Council of Canada (NSERC) and the Genome BC-CDRD Innovation Fund for financial support. R.N.Y. acknowledges the support of Simon Fraser University, the British Columbia Government Leading Edge Endowment Fund and Merck Canada Inc.

Abbreviations:

BHI	brain–heart infusion broth
MRSA	methicillin-resistant *Staphylococcus aureus*
PK	pyruvate kinase
MIC	minimal inhibitory concentration
TSA	tryptic soy agar
TSB	tryptic soy broth

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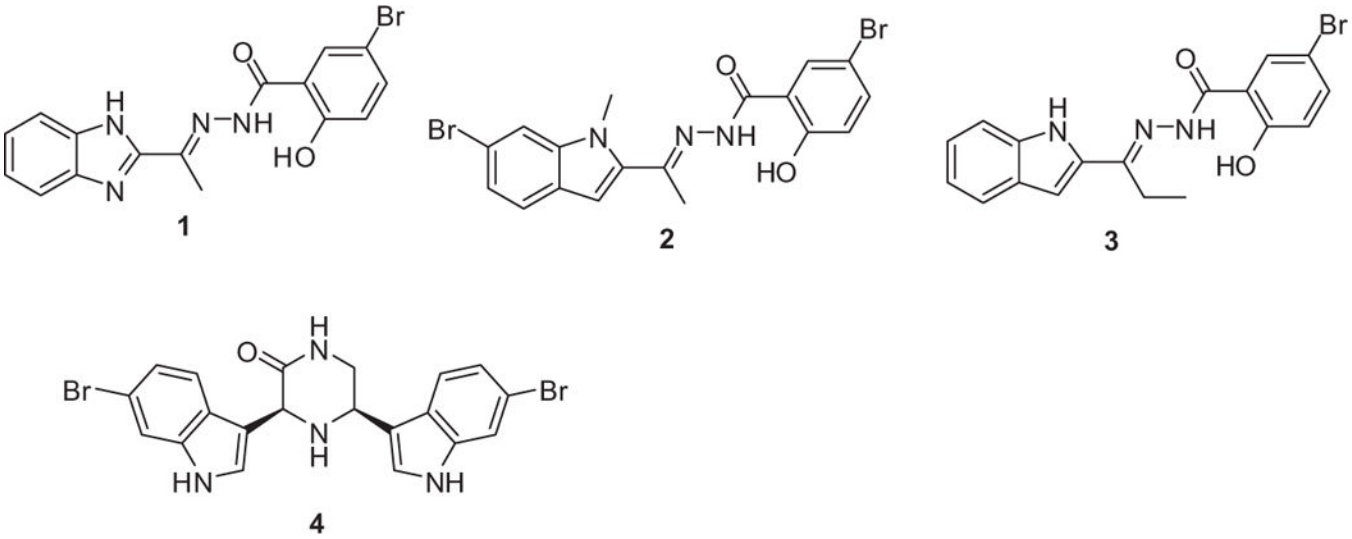


Figure 1.
MRSA PK inhibitors **1–4**.

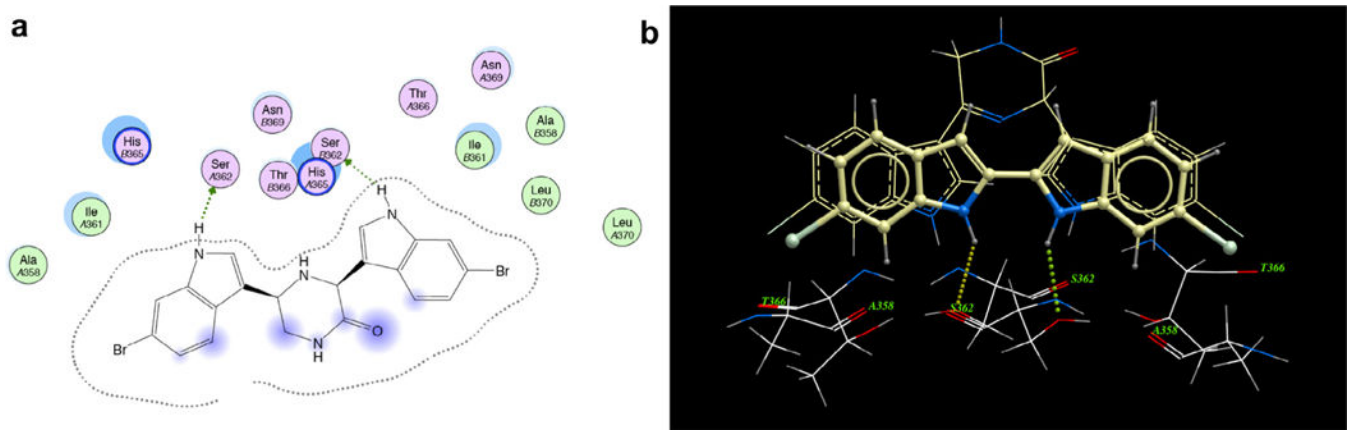


Figure 2.

Binding mode of compound **4** at the MRSA PK tetramer interface binding site; (a) a two-dimensional map (MOE software) of the binding interactions between **1** and the interface site based on its co-crystallization with MRSA PK. Green arrows depict hydrogen-accepting interactions between **4** and MRSA PK residues from the interface (left). (b) Modeled overlay of compound **10b** with **4** in the MRSA binding site (right) (ICM software from PDB data file 3t07).

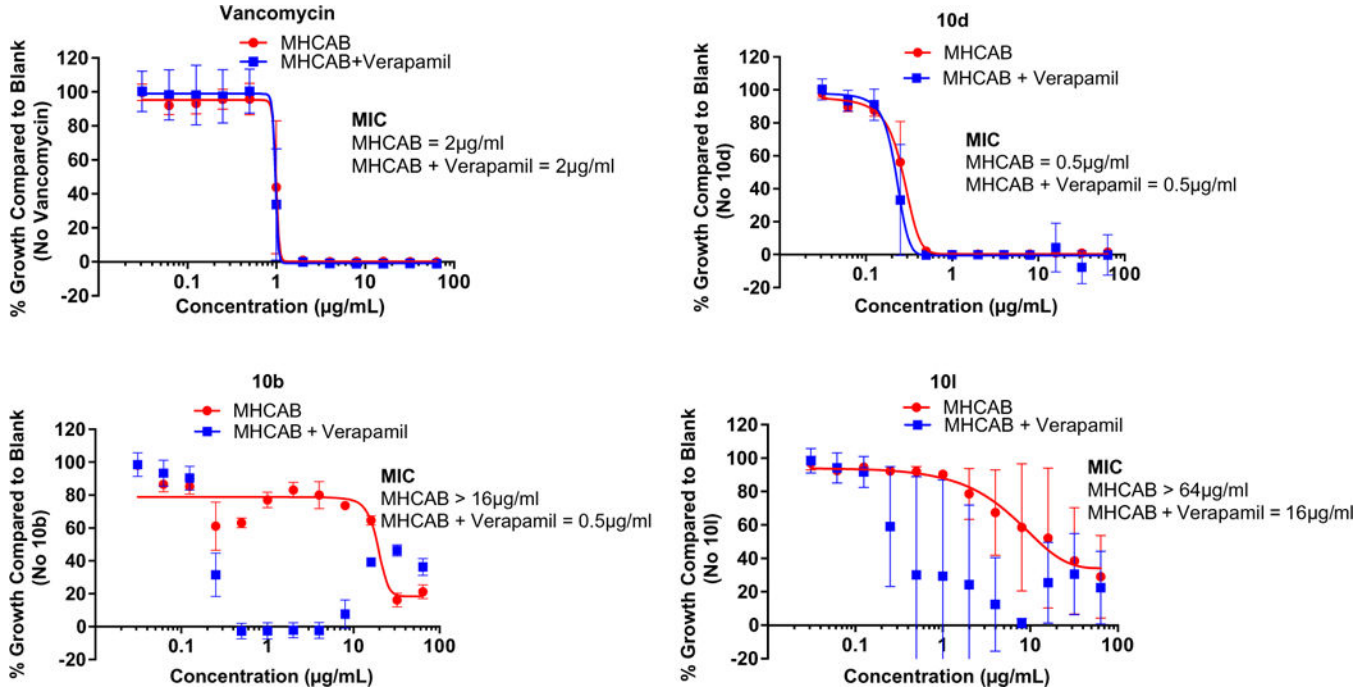


Figure 3.

The MIC determinations of **10d**, **10b** and **10l** in the presence and absence of efflux inhibitor Verapamil with *S. aureus* (ATCC29213). Assay of vancomycin, **10d** and **10b** was performed in triplicate. Compound **10l** was performed in duplicate.

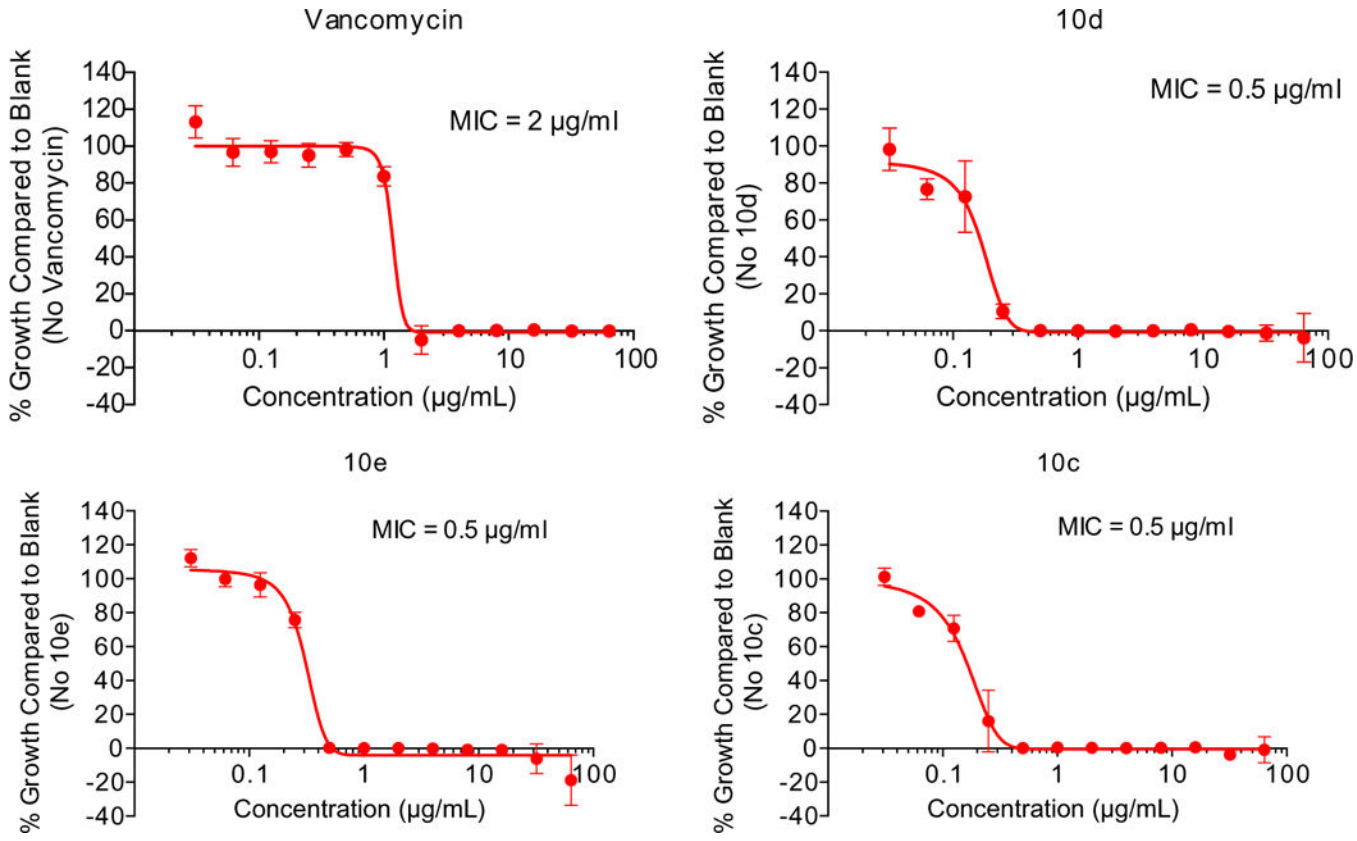


Figure 4.
MIC determinations of **10c**, **10d** and **10e** with MRSA strain MW2 (USA 400).

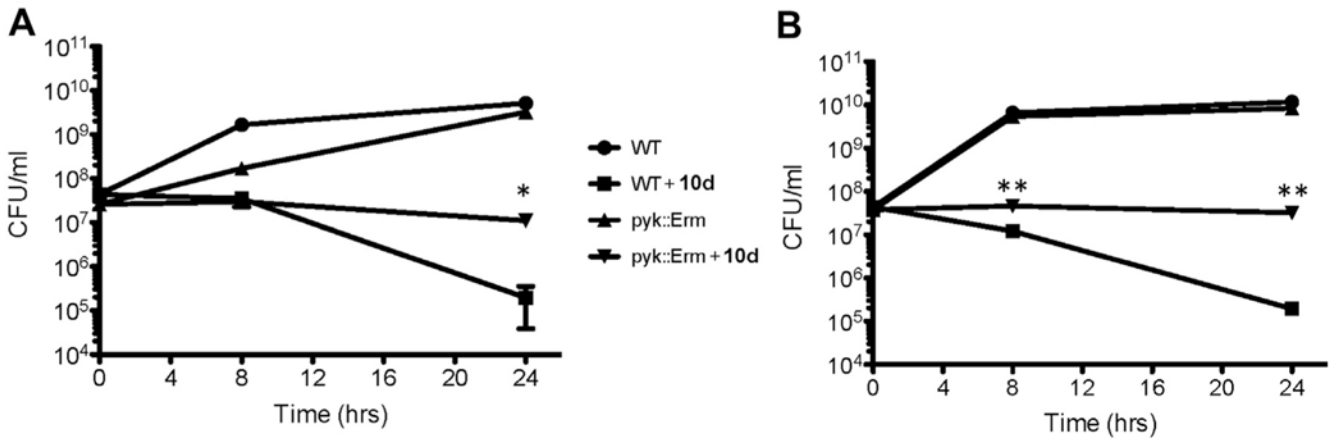
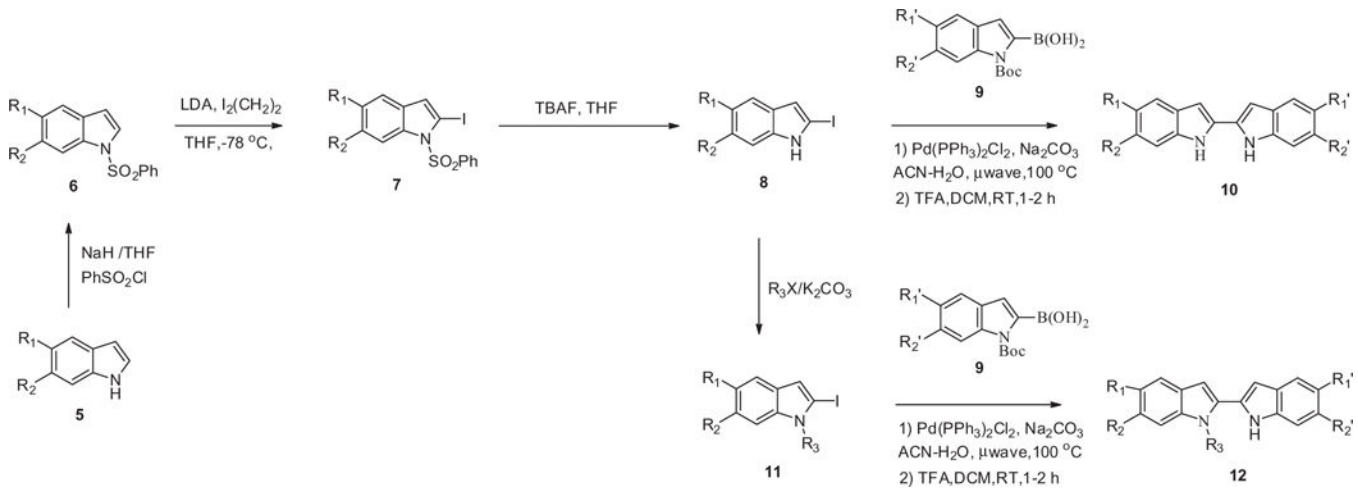


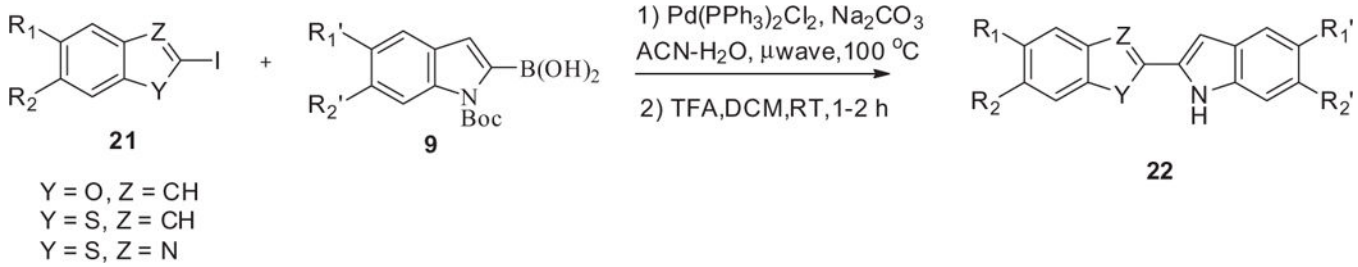
Figure 5.

Resistance of *S. aureus* LAC Δ*pyk*::Erm^R to killing by compound **10d**. (A) Resistance of *S. aureus* LAC Δ*pyk*::Erm^R to killing by 1.6 μM 10d during aerobic growth in BHI. (B) Resistance of *S. aureus* LAC Δ*pyk*::Erm^R to killing by 1.6 μM **10d** during aerobic growth in TSB without glucose supplemented with 1% sodium pyruvate. Significance was assessed between WT LAC and Δ*pyk*::Erm^R LAC treated with **10d** at 8 and 24 h using a Students two-sided t-test (*p-value ⩽0.05, **p-value ⩽0.0001). Error bars = ±SD. (n = 3).

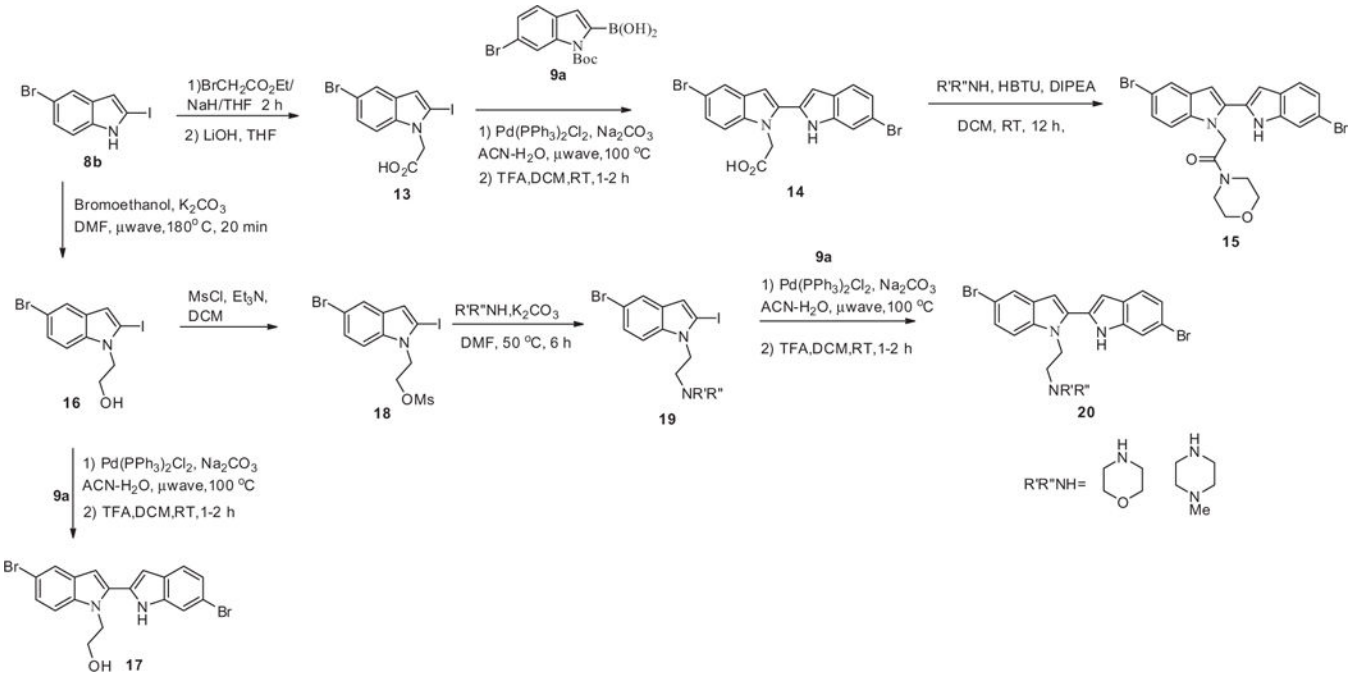


Scheme 1.

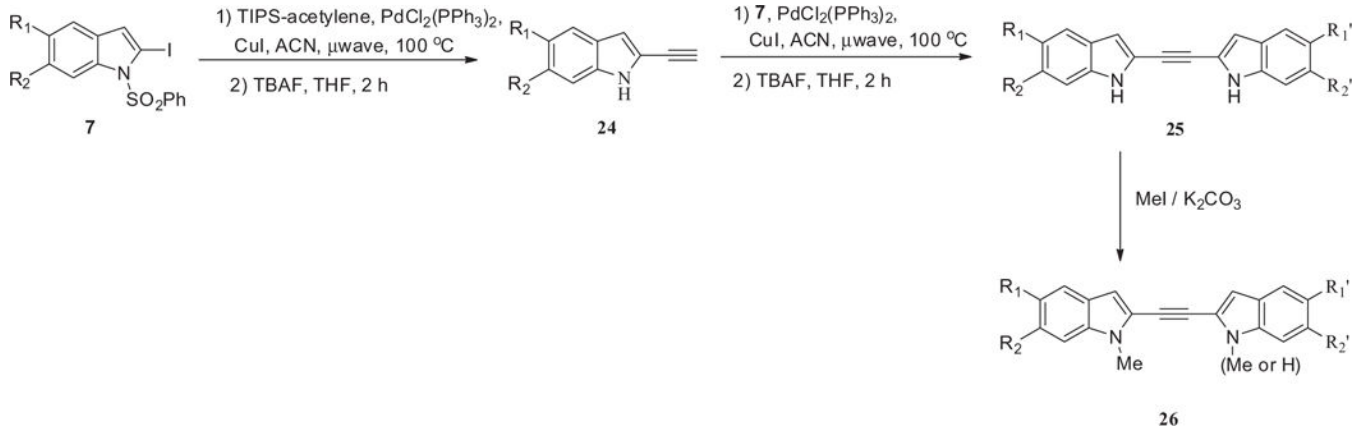
General synthesis of bis-indoles **10** and **12**.



Scheme 2.
General synthesis of mono-indole coupled with heterocycles.

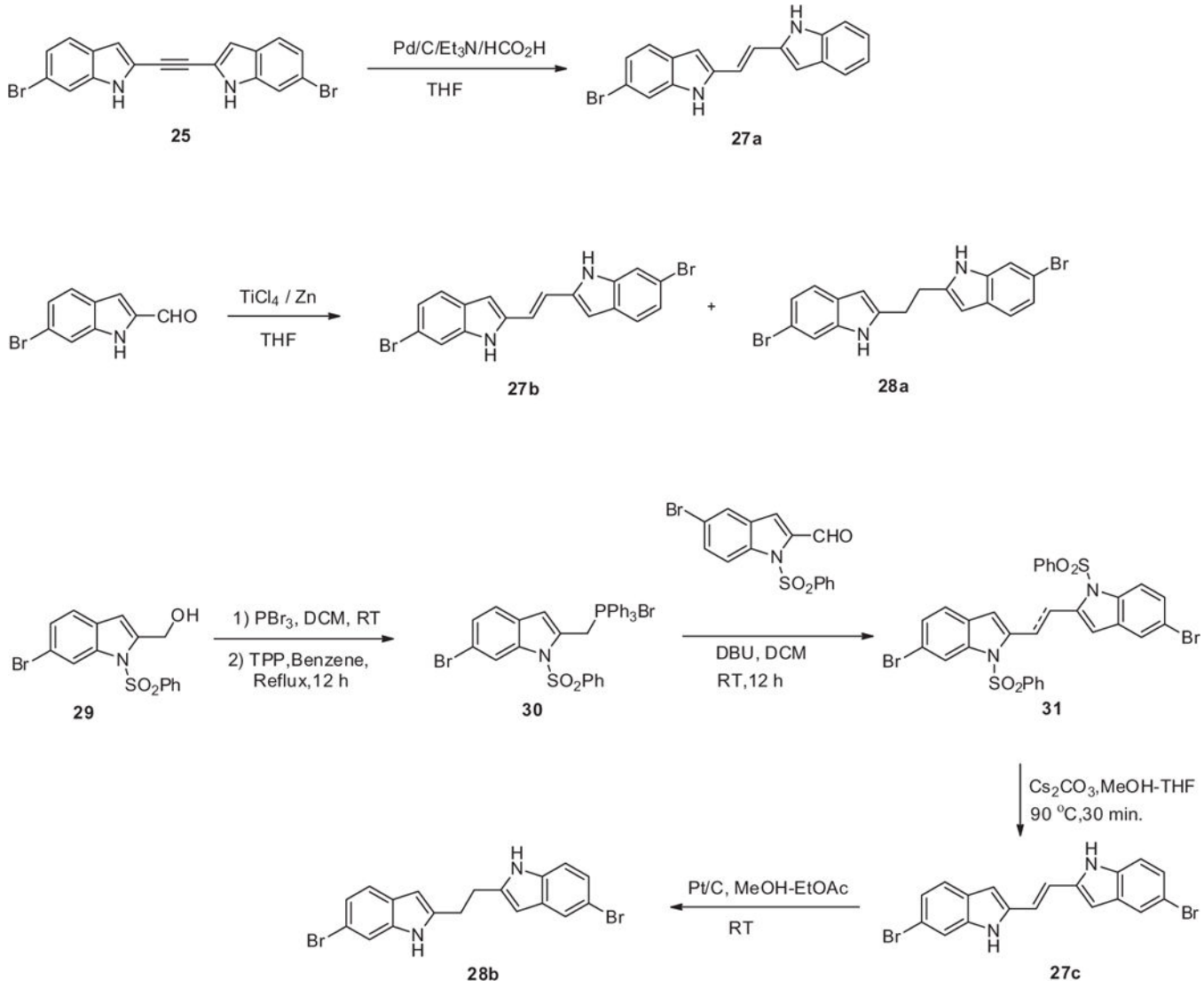


Scheme 3.
General synthesis of bis-indoles **14, 15, 17** and **20**.

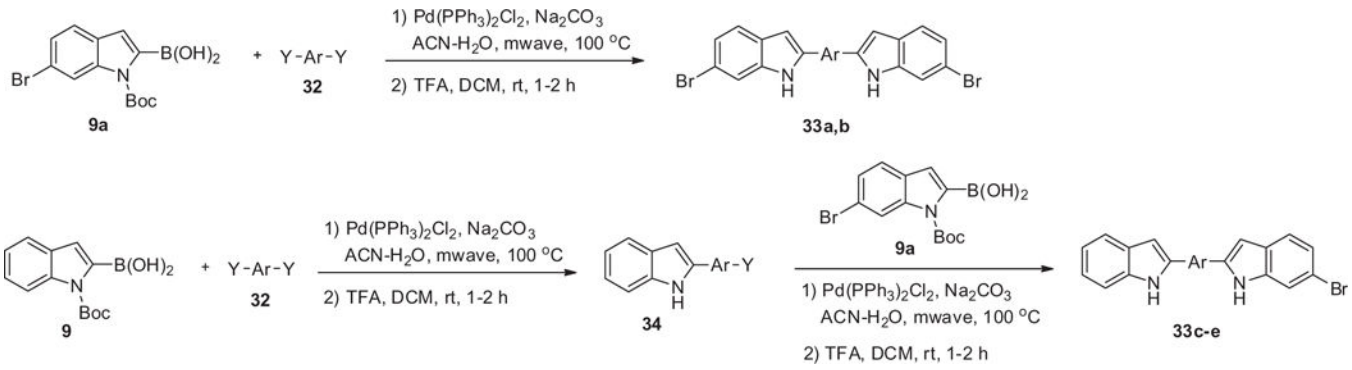


Scheme 4.

General synthesis of bis-indoles with acetylene linker.



Scheme 5.
General synthesis of bis-indoles **27** and **28**.

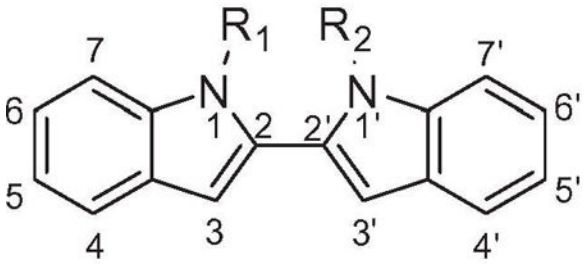


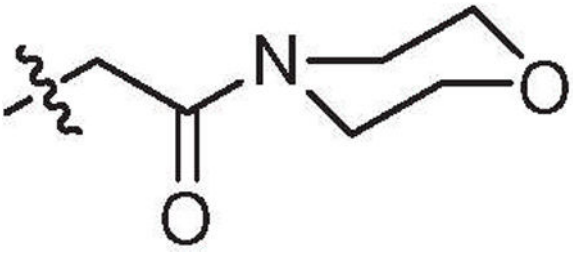
Scheme 6.

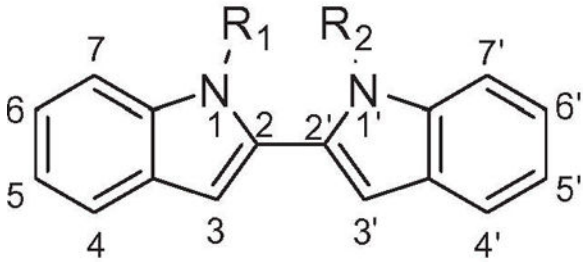
General synthesis of bis-indoles with an aryl linker.

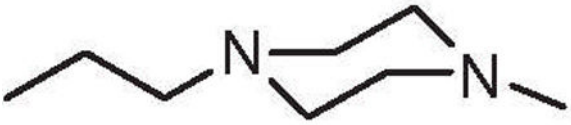
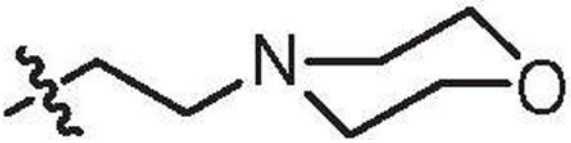
Table 1

PK inhibitory activity and antimicrobial activity of substituted bis-indoles



Analogs	IC_{50} [a] (nM)	MIC [b] (μg/ml)	R_1	R_2	Substitutions
10a	21.4	2.0	H	H	6-Bromo
10b	7.0	16.0	H	H	6,6′-Dibromo
10c	2.2	0.3 (3)	H	H	6,5′-Dibromo
10d	2.0	0.3	H	H	6-Bromo-5′-chloro
10e	2.8	0.3	H	H	6-Bromo-5′-fluoro
10f	2.5	>64	H	H	6-Bromo-5′-methoxy
10g	2.2	>64	H	H	6-Bromo-5′-phenyl
10h	36% @ 10 μM	ND	H	H	5-Bromo
10i	3.0	>64 (2)	H	H	5,5′-Dibromo
10j	39% @ 10 μM	ND	H	H	5,6-Dibromo
10k	1.5	0.5	H	H	5,6,6′-Tribromo
10l	2.0	>64	H	H	5,5′,6,6′-Tetrabromo
10m	1.6	1.0	H	H	5,6,5′-Tribromo
12a	1.0	>64 (2)	H	CH_3	6,5′-Dibromo
12b	25% @ 10 μM	ND	CH_3	H	6,5′-Dibromo
12c	1.9	>64	H	CH_2OCH_3	6,5′-Dibromo
14	11.1	>64	H	CH_2COOH	6,5′-Dibromo
15	6.0	>64	H		6,5′-Dibromo
17	1.3	2.0	H	CH_2CH_2OH	6,5′-Dibromo



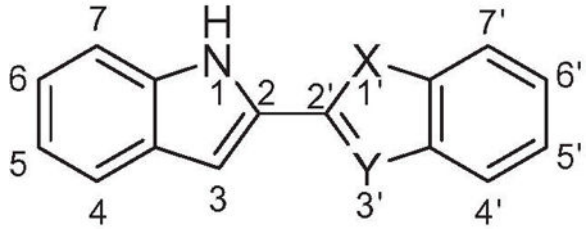
Analogs	IC_{50}[a] (nM)	MIC[b] (μg/ml)	R_1	R_2	Substitutions
20a	2.0	4.0	H		6,5′-Dibromo
20b	1.2	>64	H		6,5′-Dibromo

[a] IC_{50} values are calculated from a triplicate 15-point titration. Alternatively the % inhibition at the highest concentration tested is presented

[b] Minimum concentration to give >98% inhibition of growth of *S. aureus* ATCC 29213 (single determination or average of (*n*) determinations). Control MIC (vancomycin) is 1 μg/ml.

Table 2

PK inhibitory activity and antimicrobial activity of compounds **22a–g**



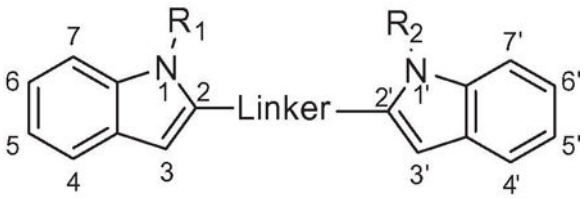
Analogs	IC_{50}[a] (nM)	MIC[b] (μg/ml)	X	Y	Substitutions
10a	21.4	2.0			
22a	151.7	>64	S	N	6-Bromo
22b	107.6 (2)	>64	O	CH	6-Bromo
22c	14.0	>64	S	CH	6-Bromo
22d	358.1	>64	S	N	5-Bromo
22e	45% @ 10 μM	ND	O	CH	5-Bromo
22f	42% @ 1 μM	>64	S	CH	5-Bromo
22g	4.1	>64	S	N	5,6′-Dibromo

[a] IC_{50} values are calculated from a triplicate 15-point titration or are an average of (*n*) such determinations as indicated. Alternatively the % inhibition at the highest concentration tested is presented.

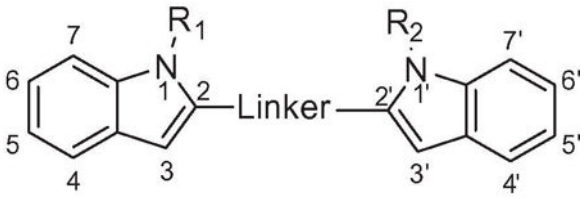
[b] Minimum concentration to give >98% inhibition of growth of *S. aureus* ATCC 29213 (single determination). Control MIC (vancomycin) is 1 μg/ml.

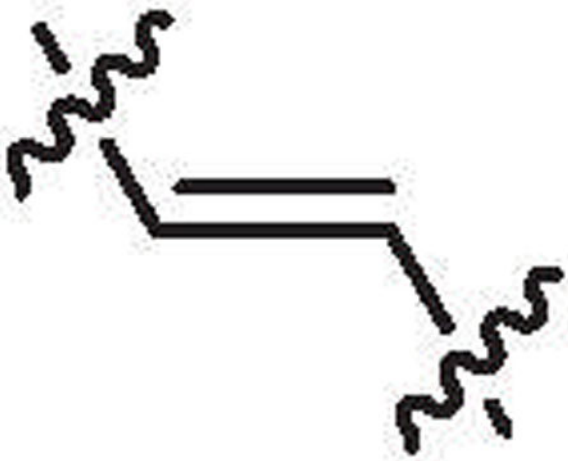
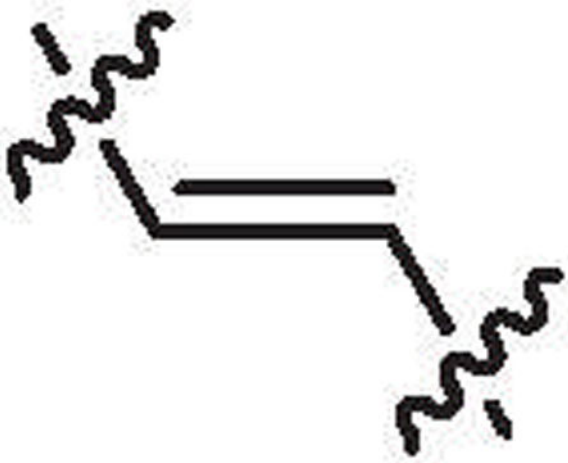
Table 3

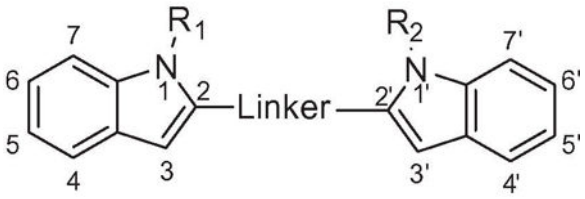
PK inhibitory activity and antimicrobial activity of compounds with modification on the linker

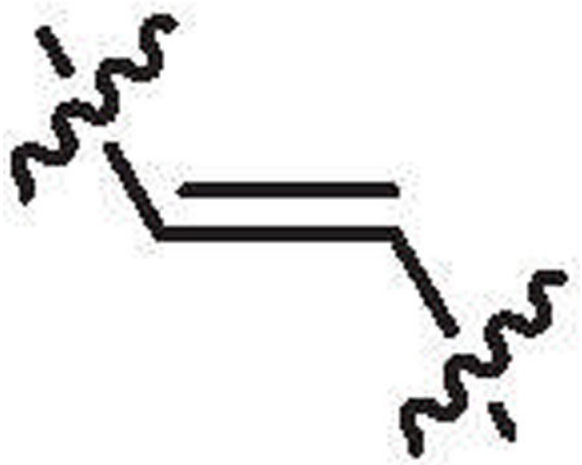
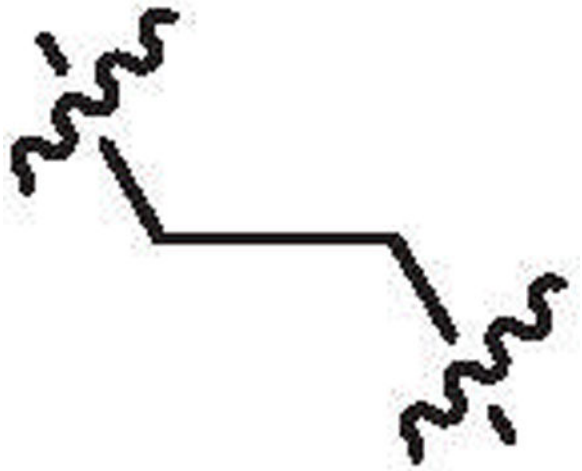


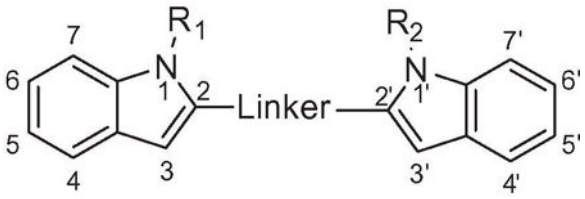
Analogs	IC_{50} [a] (nM)	MIC [b] (μg/ml)	Linker	R_1	R_2	Substitutions
10a	21.4	2.0				
25a	7.1	>64		H	H	6-Bromo
25b	2.4	>64		H	H	6,6′-Dibromo
25c	4.7	16		H	H	6,5′-Dibromo
26a	13.7	>64		CH_3	H	6,6′-Dibromo

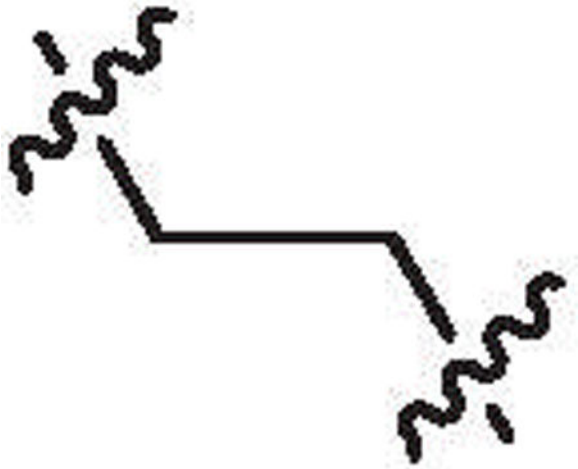


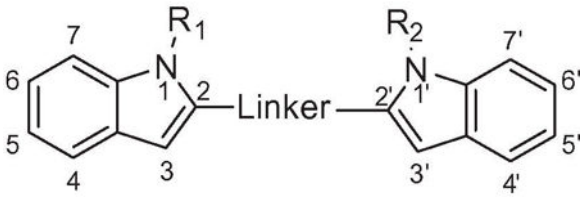
Analogs	IC_{50} [a] (nM)	MIC [b] (μg/ml)	Linker	R_1	R_2	Substitutions
26b	27% @ 10 μM	ND		H	CH_3	6,6′-Dibromo
27a	4.5	>64		H	H	6-Bromo
27b	3.9	>64		H	H	6,6′-Dibromo

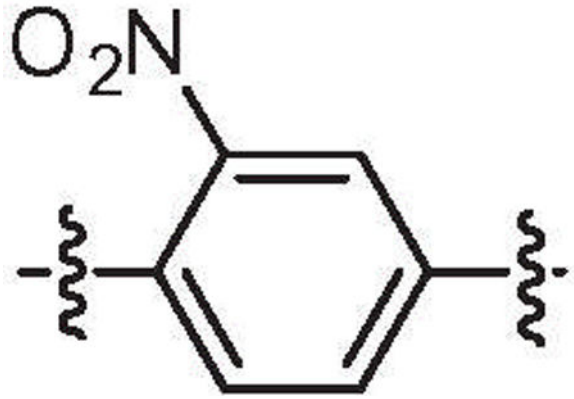
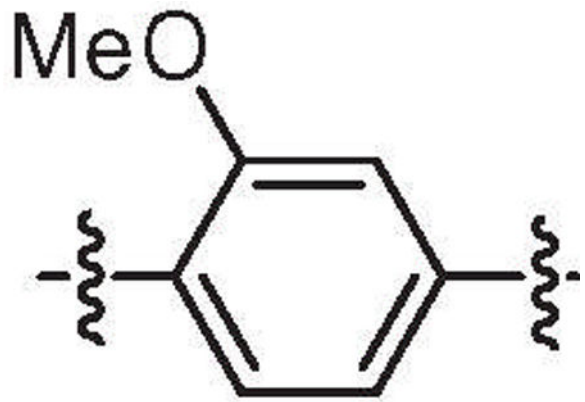
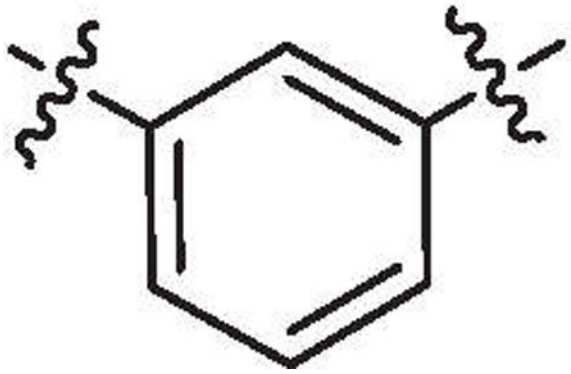


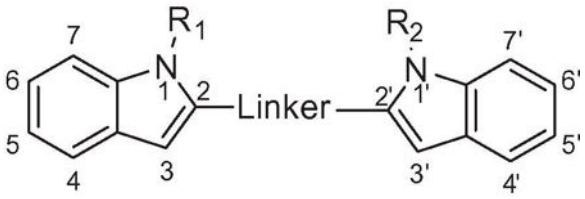
Analogs	IC_{50} [a] (nM)	MIC [b] (μg/ml)	Linker	R_1	R_2	Substitutions
27c	4.1	>64		H	H	6,5′-Dibromo
28a	6.5	>64		H	H	6,6′-Dibromo

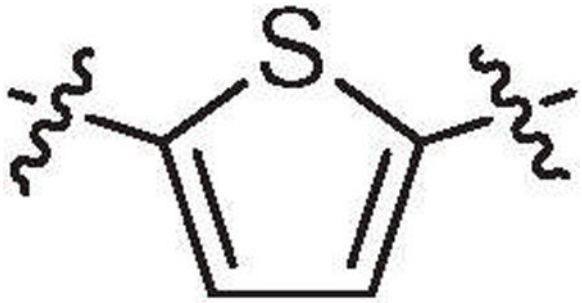


Analogs	IC_{50} [a] (nM)	MIC [b] (μg/ml)	Linker	R_1	R_2	Substitutions
28b	6.3	>64		H	H	6,5′-Dibromo
33a	54% @ 1 μM	>64		H	H	6,6′-Dibromo
33c	5.6	>64		H	H	6-Bromo



Analogs	IC_{50} [a] (nM)	MIC [b] (μg/ml)	Linker	R_1	R_2	Substitutions
33d	1.8	2.0		H	H	6-Bromo
33e	2.1	>64		H	H	6-Bromo
33f	36% @ 10 μM	ND		H	H	6-Bromo



Analogs	IC_{50} [a] (nM)	MIC [b] (μg/ml)	Linker	R_1	R_2	Substitutions
33b	28% @ 1 μM	>64		H	H	6,6′-Dibromo

[a] IC_{50} values are calculated from a triplicate 15-point titration. Alternatively the % inhibition at the highest concentration tested is presented.

[b] Minimum concentration to give >98% inhibition of growth of *S. aureus* ATCC 29213 (single determination). Control MIC (vancomycin) is 1 μg/ml.

Table 4

Inhibition of enzymatic activities of MRSA PK and human PK isoforms at 10 μM

Analogs	% Inhibition of MRSA PK	% Inhibition of human M1	% Inhibition of human M2	% Inhibition of human R	% Inhibition of human L
10b	84.9 (2)	10.3 (2)	0.1 (2)	18.3 (2)	7.9 (2)
10c	97.8	6.9	8.7	13.6	6.1
10i	71	12.3	0.5	24.4	9.8
10k	94.1	8.6	15.8	33.4	23.7
10l	97.6	11.1	2.3	20.7	8.2
12a	99.0	13.9	9.1	2.7	2.8
17	100.9	14.6	12.1	25.2	6.8
20a	100.1	3.2	1.7	26.1	16.8
20b	100.6	13.1	3.5	50.7	32.1
22a	89.8	12.4	3.0	21.6	7.8
22b	95.9	5.3	0.4	−5.5	−6.0
25c	86.3	19.5	8.7	27.8	14.0
27a	93.4	16.1	−0.9	23.8	12.9
28a	93.4	9.9	1.3	24.1	9.7
33d	86.2	9.5	6.8	0.0	−8.9
33e	62.2	7.3	14.6	13.5	4.3

Table 5

Mammalian cell cytotoxicity of selected compounds

Analogues	% Cell viability found on incubation with HEK 293 cell line		
	1 μg/ml	10 μg/ml	100 μg/ml
10b	103	106	118
10f	107	88	72
10k	102	71	63
12c	123	102	73
27a	107	114	115
28a	104 (2)	92 (2)	85 (2)