Received Date : 15-Oct-2014

Revised Date : 22-Dec-2014

Accepted Date : 06-Jan-2015

Article type : Research Letter

Design and biological evaluation of furan/pyrrole/thiophene-2-carboxamide derivatives as efficient DNA gyraseB inhibitors of *Staphylococcus aureus*

Renuka Janupally[a], Bhramam Medepi[a], Parthiban Brindha Devi[a], Priyanka Suryadevara[a], Variam Ullas Jeankumar[a], Pushkar Kulkarni[b,c], Perumal Yogeeswari[a], Dharmarajan Sriram[a]*

[a]*Department of Pharmacy, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Shameerpet, Jawahar Nagar, Ranga Reddy District, Hyderabad 500078, Andhra Pradesh, India.*

[b]*Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India.*

[c]*Zephase Therapeutics (An Incubated Company at the Dr. Reddy's Institute of Life Sciences), University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India.*

DNA topoisomerases are well validated targets in microorganisms. DNA gyraseB is one of the most important enzyme among them as per their clinical importance. In earlier study, a novel lead 4-((4-(furan-2-carboxamido)phenyl)amino)-4-oxobutanoic acid, was identified as inhibitor aganist DNA gyraseB with an IC_{50} of 12.88±1.39 µM. Subsequently, analogues of this lead were developed and evaluated through *in vitro* assays and *in vivo* studies. Among the 24 analogues, compound **22** was found to be the top hit with an improved DNA gyraseB activity of 5.35±0.61 µM, the binding affinity of this compound was further ascertained biophysically through differential scanning fluorimetry. The most potent ligand did not show any signs of cardiotoxicity

in zebra fish ether-ago-go-related gene (zERG), ascertaining the safety profile of this series a breakthrough among the previously reported cardiotoxic gyraseB inhibitors.

Key words: *Staphylococcus aureus*, DNA gyrase, cytotoxicity, biofilm.

Abbreviations: e-pharmacophore, energy based pharmacophore; MRSA, methicillin resistant Staphylococcus aureus; zERG, Zebra fish ether-ago-go-related gene.

*Corresponding author: D. Sriram (dsriram@hyderabad.bits-pilani.ac.in)

Occurrence of increasingly serious threat to global public health is a consequence of antimicrobial resistance, which threaten the effective prevention and treatment of an increasing range of infections caused by the bacteria (1). *Staphylococcus* aureus, a gram positive bacteria a normal flora, inhabiting 30% of human population, had emerged as a causative agent of many pathogenic infections globally, a major cause of morbidity and mortality in community associated environments and hospital environment (2-3). As expected, resistance to first-line drugs to treat infections caused by *Staphlylococcus aureus* is also increasing alarmingly. However development of methicillin resistant *Staphylococcus aureus* (MRSA- multidrug-resistant) strain in the early 1960s, re-urged the necessity of novel drugs against this dreadful bacteria (4). Presently, vancomycin is the only available source to treat *S. aureus* infections to certain extent. One of the important target clinically validated as bactericidal in *S. aureus* was DNA topoisomerase II and

IV. DNA gyrase belongs to DNA topoisomerase II that is required to maintain the DNA topology during replication, transcription in bacteria. It consists of two A subunits and two B subunits attached to form a holoenzyme (5). For the bacterial cell to survive, the DNA as to exist in a negative supercoiled form. The functional enzyme performs the negative supercoiling of the DNA. The dimeric subunit A involves in the cleavage and religation of the DNA while the B subunit aids in the catalytic ATP hydrolysis. As fluoroquinolones have almost become ineffective due to fast emerging Staphylococcal strains, caused due to alteration in the interaction of the fluoroquinolones with their target sites and also due to alterations that affect drug (6). The other drawback of fluoroquinolones is that they are more potent against the gram-negative bacteria when compared with gram-positive ones like *S. aureus* due to the presence of another homologous target topoisomerase IV (7). Furthermore, research groups from both industry and academia focused on the development of antibacterial DNA gyraseB inhibitors against various organisms have identified many potent inhibitors belonging to the chemical class of indolinones (8), pyrimidines (9), indazoles (10), rhodanines (11), pyrazolothiazoles (12), pyrrolopyrimidines (13), pyrrolamides (14) as potential hits, though clinically very little has been achieved when it comes to Staphylococcal gyrase B inhibitors. Keeping this in view, we designed novel class of furan/pyrrole/thiophene-2-carboxamide derivatives molecules, targeting DNA gyraseB domain. In the present study, we explored furan/pyrrole/thio-phene-2-carboxamide derivatives and their inhibitory profiles against DNA gyrase, cell cytotoxities and zERG activity as well to know the overall effect of these compounds biologically.

As reported in our earlier publication (2), twelve compounds were procured from Asinex database through e-pharmacophore based screening approach using PDB Id: (3TTZ). Among the promising *in silico* results of the twelve virtual screening hits obtained, compound **5** (considered

as lead in this series) was one among them with *S. aureus* DNA gyraseB activity of 12.88±1.39 µM and an MIC of 79.03 µM. **Figure 1** depicts the structure of the lead compound **5**. Considering this compound as a lead in this series, twenty three analogues were synthesized with the goal of obtaining a potent compound with tractable SAR and potencies better than the identified initial screening hit. Based on the potency and synthetic feasibility; a series of twenty three analogues were designed and synthesized around the lead molecule **5** in the ligand expansion step, to trace the structure activity relationship. Considering the important interaction that the *p*-phenylenediamine linker retained with Asp 81, it was decided to retain the linker and explore the right and left hand core as shown in **Table 1.** The synthetic pathway used to assemble library has been depicted in **Scheme 1**. The first step involved the selective protection of *p*-phenylenediamine using Boc anhydride under controlled conditions by a previously reported protocol. The monoprotected linker was then functionalized with various substituted heterocyclic acids to give the corresponding amide derivatives in excellent yields. Further de-protection and treatment with various anhydrides gave the corresponding acid analogues in moderate yield. The corresponding ester and hydrazide derivative of the acid analogue were also attempted in synthesis, to understand the role of this group in activity determination.

The DNA gyraseB assay was performed as per the protocol (14-15). Absence of highly active DNA gyraseB ATPase activity of the purified *S. aureus* gyraseB *in vitro* like other bacteria made us use the E. coli DNA gyraseA for enhancing the activity of *S. aureus* gyraseB (2). Subsequently, *E. coli* DNA gyraseA in combination with *S. aureus* DNA gyraseB was used to do the ATPase activity. As the reference ligand **5** from Asinex database (BAS04380545) showed an IC_{50} of 12.88±1.39 µM search was made to obtain a potent ligand with better inhibitory profile, this compound was found to be in hydrogen bonding with residues Gly85 and Arg144 as shown

in **Figure 2**. Also a *cation-pi* interaction was present with Arg81 and strong hydrophobic interactions by the furan ring with Ile51, Val79, Ile86 and Ile175. Hence, based on these interactions, the compound was further modified and derivatized placing the substituents majorly at three positions – substituents over five membered ring (R_1), substituents at oxygen in furan ring (X) and final substitution at the terminal hydroxyl group (R_2). The synthesized compounds were docked with the crystal structure of gyraseB of *S. aureus* (PDB code–3TTZ) so as to observe the mode of interaction of the compounds at the active pocket and to determine its orientation *in silico*, subsequently the biological activity too was performed with different drug concentrations starting from a range of 100 µM to 1.56 µM. Fourteen compounds showed greater than 50% inhibitions at 100 and 50 µM concentration of the drug concentration. Further, all these molecules were tested at lower drug concentrations of 25, 12.5, 6.25, 3.12 and 1.56 µM. Initially, substitutions were made at R_2 position replacing hydroxyl group with methoxy **13**, and hydrazine **21**. These compounds were found to be inactive with docking score of -3.9 and -4.1 kcal/mol respectively, with gyraseB assay values ranging from 27-36 µM suggesting the favorability of hydroxyl group for the activity as in **5**. Hydroxyl group was involved in hydrogen bonding with Arg144, an important active site residue, deletion of which was found to deplete the activity. The next substitution was done at both R_1 and R_2 positions, where R_1 is occupied by 2-methyl moiety and the R_2 position with hydroxyl **6**, methoxy **14** and hydrazine **22** groups. Of these, compound **22,** N-(4-(4-hydrazinyl-4-oxobutanamido) phenyl)-5-methylfuran-2-carboxamide emerged as the top hit molecule with a docking score of -8.2 with a fitness value of 1.9, a DNA gyraseB IC_{50} of 5.35±0.44 µM almost one half of the lead compound **5**, calculated by GraphPad Prism software and an MIC of 18.92 µM. The dose response curve for this compound is shown in **Figure 3**. The binding profile of this compound revealed the presence of four hydrogen bonds at active site with

Val79, Asp81, Gly85 and Thr173 as shown in **Figure 4**. It was found that the presence of hydrazine group at R_2 position contributed for three hydrogen bonds and made the compound orient towards the hydrophobic pocket adding up to non-polar interactions. The compounds were re-screened for the final time in triplicates to ensure their activity results. The other two molecules were found to be orienting totally in opposite direction shown as shown in supporting information **Figure S1.**

The substitutions were further carried out using nitro at R_1 position and the same hydroxyl in **7** with docking score of -4.6, methoxy group in **15** with docking score of -4.3 and hydrazine **23** group at R_2 position with docking score of -4.9. These compounds were found to be inactive against gyraseB (IC_{50} around 27 – 40 μM). The docking studies of these compounds revealed their orientation with nitro group facing the hydrophobic pocket making it impossible to involve in any hydrophobic contacts resulting the compounds inactivity.

The further substitutions were done by replacing the furan ring with thiophene in **8, 16** and **24** and 2-methyl thiophene in **9, 17** and **25** with the same three substitutions at R_2 position. All these compounds were found to be inactive (IC_{50} ranging 21 – >40 μM) except compound **25** with a docking score of -7.3 and a fitness value of 1.9. Potency of this compound (IC_{50} – 9.711 μM and MIC – 18.04 μM) may be attributed to the presence of hydrazine which was found to be participating in hydrogen bonds with Val79, Asp81 and Thr173 and the carbonyl oxygen next to thiophene ring was found to be in polar contact with Arg144 which were lacking in the remaining compounds as shown in supporting information **Figure S2.** The ethylene linker was found to be involved in strong hydrophobic contacts with Ile51 and Ile175 and thiophene ring with Pro87.

The replacement of hydrazine in compound Compounds **9** and **17** resulted in loss of activity up to five folds showing up the importance of hydrazine for *S. aureus* gyraseB.

Substitution of furan with 2-nitro thiophene and same three R_2 substitutions in **10, 18** and **26**, yielded compound **18** as active one with IC_{50} of 12.49 μM in gyraseB assay and a docking of -7.8 and a fitness value of 1.7. This may be reasoned for the for the hydrogen bond interactions with Asp81 and Arg144. The main reason can be given by non-polar interactions with thiophene ring at the hydrophobic pocket with Ile51 and Ile175. The other two compounds were not involved in hydrogen bonding with Asp81, an important active site residue, as the substitutions at R_2 position oriented the molecules away from Asp81.

The final substitutions were done with tetra hydro furan in **11, 20** and **28** and pyrrole ring in **12, 19** and **27** at R_1 position with the same R_2 substitution compounds. Compounds **11** and **20** were found to be active with docking scores of -7.3 and -7.7, with two hydrogen bonds Asp81 and Arg144 and the hydrophobic interactions with tetra hydro furan ring. Compound **28** was inactive with a docking score of -3.9 which can be explained by the reversal of binding pattern with the addition of hydrazine as the R_1 position was towards solvent which decreases the activity. All the pyrrole substituted compounds were found to be highly inactive suggesting the un-favorableness of pyrrole ring as it was weakly involved in hydrophobic interactions. All the pyrrole substituted compounds were found to be inactive suggesting the moiety unfavourable for gyraseB protein.

To sum up, it was observed that not all substitutions were favourable for *S. aureus* gyraseB activity. Some of the key features of compounds that are crucial for activity may be – (i) a hydrophobic ring (like furan, thiophene, pyrrole) at one terminal of compound is important for the

activity and (ii) a combination of substitutions like methoxy over furan or thiophene at one terminal and hydrazine (-NH-NH_2) at the other terminal of the compound is highly fruitful as observed in compounds **22** and **25**. Compounds with such above features can be desirable in drug development process for *S. aureus* targeting gyraseB. Novobiocin, an aminocoumarin was considered as a standard compound in this assay with an IC_{50} of 0.125 ± 0.24 µM.

Subsequently, all the molecules were screened for the DNA supercoiling assay as well to confirm the inhibitory profiles of the gyraseB inhibitors (2, 16) *S. aureus* DNA supercoiling kit was procured (Inspiralis Pvt. Limited, Norwich) and the different range of drug concentrations were used from 100 to 1 µM. Compounds **9, 6, 7, 25, 10, 20** and **11** showed better inhibitory profiles with an IC_{50} of less than 10 µM. While the reference compound **5** had an IC_{50} of 3.12±1.6 µM, the synthesized compound **22** had a better IC_{50} of 2.79±0.59 µM. In this assay too novobiocin was considered as a standard inhibitor with an IC_{50} of 0.030 ± 0.01 µM. The dose dependent inhibitor profile is shown in **Figure 5.**

Twenty four synthesized compounds were further screened for their *in vitro* antibacterial activity against two bacterial strains of *Staphylococcus aureus*, namely *S. aureus* MTCC 3160 and MRSA 96. The assay was performed according to the Clinical and Laboratory Standards Institute (17) using the protocol as described in supporting information. The concentration of the drugs used was in a range of 256-0.75 µg/mL. While almost all the compounds showed an MIC less than 100 µM, in MTCC 3160 strain, they showed two-three fold higher MIC values in MRSA 96 strain elucidating the drugs efficiency to inhibit the multi-resistant strain as well (2). As per the table, compounds **22** and **17** showed better MIC values at 18.92 µM and 16.56 µM almost one-

fourth less concentration when compared to the lead **5**. Ofloxacin and ciprofloxacin were included as reference compounds in the assay. The overall results of the antibacterial screening are presented in **Table 1**. Few compounds **6, 10, 27, 5, 24, 9, 19, 12** and **28** exhibited weak antimicrobial activities which could be attributed to their permeability or efflux pump transportation hindrances.

All the compounds were also tested for their biofilm inhibition studies too. Being an extracellular polysaccharide, produced by *S. aureus* species, biofilm aids in attachment and matrix formation around the bacteria that result in the alteration in the phenotype of the organism, resulting in the development of resistance towards the drugs. According to the recent statistics, it was estimated that 65% of all the human bacterial infections were due to biofilm formation (18) moreover people with MRSA infections are estimated to be 64% more likely to die than people with a non-resistant form (1). While the emergence of greater resistance of *S. aureus* biofilms against the antibacterial agents was attributed to failure of antibiotics to penetrate the biofilm, the other important parameter being the existence of different metabolic states of the cells in the biofilm aggregates associated with differential expression of genes by the bacteria concerned in the same biofilm aggregates (19). All the synthesized compounds were screened for inhibition of *S. aureus* biofilm formation in a strong biofilm producer MRSA 96 strain. The extent of inhibition and the efficiency of the drugs were monitored quantitatively and qualitatively by the Congo red method (20). Assays were performed in a 96 flat-bottomed well plates at different drug concentration range starting from a high 250 to 0.75 µM. While the potent ligand **22** showed an IC_{50} of 18.92 µM, all the compounds except **21**, showed an IC_{50} less than 100 µM indicating the efficiency of these series of compounds towards inhibition of biofilm formation.

Furthermore, all the compounds were tested for their eukaryotic safety profile information. The compounds were screened for their *in vitro* cytotoxicity against mammalian RAW 264.7 cell line at 100 µM concentration in triplicates by utilizing (4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (21-22). It was observed that all the twenty four compounds inhibitory range was below 25 µM. The most potent compound **22** showed only 9.35% cytotoxicity at the highest concentration tested which was on par with novobiocin standard which had 11.4%.

We tried to test the potent ligand **22** in an *in vivo* mouse septicaemia model too. In brief, *S. aureus* inoculum of 3*10^7 cfu/mouse was administered to CD-1 female mice (10 per group) via intraperitoneal route (2). The infected mice were then treated with compound **6** in varying doses of 5, 10, and 25 mg/kg weight at time of and 4 h of post infection. Standard vancomycin was used as a control and dosed at 5 mg/kg weight. The mice survival was assessed 24 h post infection. Compound **22** was found to protect 40% of animals at 5 mg/kg, 60% and 100% protection at 10 and 25 mg/kg respectively as shown in **Figure 6.**

The fluoroquinolones, considered as the only clinically approved DNA gyrase inhibitors till date suffer from hERG toxicity mainly the potent moxifloxacin (23-24). As per literature, previously reported antibacterial C- and N-linked aminopiperidine DNA gyrase inhibitors suffered from serious hERG toxicity, we tested the top hits to ensure that the newly designed molecules did not suffer from such drawbacks. Compound **22** and **25** were subjected for hERG channel inhibition studies by assessing the arrhythmogenic potential on zebra fish ether-a-go-go-related gene (zERG) which was orthologous to the human ether-a-go-go-related gene (hERG),

due to their conserved homology. This method of assessment, possessed significant advantage over the current conventional animal models which include ethical issues, low compound requirement, manually less tedious and low cost. Furthermore, Milan et al.(25) and Mittelstadt et al. (26) had reported that bradycardia and atrio-ventricular dissociation in zebrafish larvae can be used as a surrogate marker for hERG channel inhibition thereby affecting the rapid component of the repolarizing potassium current and inducing arrhythmia in the model. In our study, compound **22** and **25** were subjected to hERG channel inhibition in concentrations ranging from 1 μM to 30 μM with 0.1% DMSO as vehicle, the heart rate variations and AV ratio were analysed by using a protocol described in more detail in supporting information. Both the compounds **22** and **25** were found to be safe when compared to the positive control (20 μM terfenadine), by not showing any significant cardiotoxicity until 30 μM concentration of the drug. Furthermore, there was no significant change in the heart rate or AV ratio, in comparison to control group making them relatively safe, a significant breakthrough when compared to otherwise cardiotoxic terfenadine and amiodarones as illustrated in **Figure 7a-7b.** The hERG figure (**S3a and S3b**) for compound **25** is shown in supporting information.

To know the thermal stability of the DNA gyraseB protein when associated with the ligand differential scanning fluorimetry (DSF) studies were performed (22, 27). The most active compound **22** from this furan/thio-phene-2-carboxamide series was subjected to DSF. Compound **22** enhanced the thermal stability of the catalytic domain of the gyraseB protein when performed in the presence and absence of the inhibitor. A significant positive shift of 2.9 ^{o}C was observed with respect to its Tm of 44 ^{o}C confirming the stability of the protein–ligand complex as shown in **Figure 8.**

A structure based e-pharmacophore model was built and based on the built hypothesis twelve molecules were shortlisted. Among them, BAS04380545, 4-((4-(furan-2-carboxamido)phenyl)amino)-4-oxobutanoic acid a compound which has been synthetically derivitized to obtain another twenty three molecules for which the *in vitro* screening and *in vivo* screening were performed. On hit expansion of this lead compound **5**, a more potent inhibitor **22** with better biological properties was obtained. Further, structural elements were identified that undoubtedly provide the basis for further optimization of the new inhibitors.

Acknowledgements

We are sincerely grateful to Prof. M. Meera and Gujjula Ravi Kumar (Sir Ronald Ross Institute of Tropical and Communicable Diseases (Nallakunta, India), Osmania University for their help with *S. aureus* MRSA 96 strain. DS acknowledges the University Grant Commission, India for a U.G.C research award, JR is thankful for CSIR for providing the financial assistance.

References

1. World health organization, Antimicrobial resistance http://www.who.int/mediacentre/factsheets/fs194/en/ (Updated April 2014).
2. Janupally R., Jeankumar V.U., Bobesh K.A., Soni V., Devi P.B., Pulla V.K., Suryadevara P., Chennubhotla K.S., Kulkarni P., Yogeeswari P., Sriram D. (2014) Structure-guided design and development of novel benzimidazole class of compounds targeting DNA gyraseB enzyme of *Staphylococcus aureus*. Bioorg. Med. Chem. S0968-0896(14)00644-0.

3. Lele A,C.; Tawari N.R.; Degani M.S. (2013) Development of pharmacophore model for *S. aureus* DNA gyrase inhibitors. Int. J Pharma Biosci. Tech. 1:34-49.

4. Rasha A., Hashem., Aymen S., Yassin., Hamdallah H., Zedan., Magdy A., Amin. (2013) Fluoroquinolone resistant mechanisms in methicillin-resistant *Staphylococcus aureus* clinical isolates in Cairo, Egypt. J Infect Dev Ctries; 11:796-803

5. Brockbank S.M., Barth P.T. (1993) Cloning, sequencing, and expression of the DNA gyrase genes from Staphylococcus aureus. J Bacteriol. 11:3269-77.

6. Ince D., Hooper D.C. (2003) Quinolone resistance due to reduced target enzyme expression. J Bacteriol. 23:6883-92.

7. Fournier, B.; Zhao, X.; Lu, T.; Drlica, K.; Hooper, D. C. (2000) Selective targeting of topoisomerase IV and DNA gyrase in Staphylococcus aureus: different patterns of quinolone-induced inhibition of DNA synthesis. Antimicrob. Agents Chemother. 8:2160-5.

8. Oblak, M.; Grdadolnik, S. G.; Kotnik, M.; Jerala, R.; Filipic, M.; Solmajer, T. (2005) In silico fragment-based discovery of indolin-2-one analogues as potent DNA gyrase inhibitors. Bioorg Med Chem Lett. 15:5207-10.

9. Lübbers, T.; Angehrn, P.; Gmünder, H.; Herzig, S.; Kulhanek, J. (2000) Design, synthesis, and structure-activity relationship studies of ATP analogues as DNA gyrase inhibitors. Bioorg Med Chem Lett. 10:821-6.

10. Boehm, H. J.; Boehringer, M.; Bur, D.; Gmuender, H.; Huber, W.; Klaus, W.; Kostrewa, D.; Kuehne, H.; Luebbers, T.; Meunier-Keller, N.; Mueller, F. (2000) Novel inhibitors of DNA gyrase: 3D structure based biased needle screening, hit validation by biophysical

methods, and 3D guided optimization. A promising alternative to random screening. J Med Chem. 43:2664-74.

11. Brvar, M.; Perdih, A.; Hodnik, V.; Renko, M.; Anderluh, G.; Jerala, R.; Solmajer, T. (2012) *In silico* discovery and biophysical evaluation of novel 5-(2-hydroxybenzylidene) rhodanine inhibitors of DNA gyrase B. Bioorg Med Chem. 20:2572-80

12. Ronkin, S. M.; Badia, M.; Bellon, S.; Grillot, A. L.; Gross, C. H.; Grossman, T. H.; Mani, N.; Parsons, J. D.; Stamos, D.; Trudeau, M.; Wei, Y.; Charifson, P. S. (2010) Discovery of pyrazolthiazoles as novel and potent inhibitors of bacterial gyrase. Bioorg Med Chem Lett. 20:2828-31.

13. Trzoss, M.; Bensen, D. C.; Li, X.; Chen, Z.; Lam, T.; Zhang, J.; Creighton, C. J.; Cunningham, M. L.; Kwan, B.; Stidham, M.; Nelson, K.; Brown-Driver, V.; Castellano, A.; Shaw, K. J.; Lightstone, F. C.; Wong, S. E.; Nguyen, T. B.; Finn, J.; Tari, L. W., (2013) Pyrrolopyrimidine inhibitors of DNA gyrase B (GyrB) and topoisomerase IV (ParE), Part II: development of inhibitors with broad spectrum, Gram-negative antibacterial activity. Bioorg Med Chem Lett. 23(5):1537-43.

14. Sherer, B. A.; Hull, K.; Green, O.; Basarab, G.; Hauck, S.; Hill, P.; Loch, J. T .; Mullen, G.; Bist, S.; Bryant, J.; Boriack-Sjodin, A.; Read, J.; DeGrace, N.; Uria-Nickelsen, M.; Illingworth, R. N.; Eakin, A. E. (2011) Pyrrolamide DNA gyrase inhibitors: optimization of antibacterial activity and efficacy. Bioorg Med Chem Lett. 21:7416-20.

15. Eakin A.; Ehmann D.; Gao N.; Karantzeni I.; Walkup G. (2004) Google Patents.

16. Dzitko K., Paneth A., Plech T., Pawełczyk J., Stączek P., Stefańska J., Paneth P. (2014) 1,4Disubstituted thiosemicarbazide derivatives are potent inhibitors of Toxoplasma gondii proliferation. Molecules. 7:9926-43.

17. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial disc Susceptibility Tests, Approved Standard M2-A9. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2006.

18. Donlan R.M. (2001) Biofilm formation: a clinically relevant microbiological process. Clin. Infect. Dis.8:1387-92.

19. Giacometti A.; Cirioni O.; Gov Y.; Ghiselli R.; Del Prete M. S.; Mocchegiani F.; Saba V.; Orlando F.; Scalise G.; Balaban. N. (2003) RNA III inhibiting peptide inhibits in vivo biofilm formation by drug-resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 6:1979-83.

20. Smith K., Perez A., Ramage G., Lappin D., Gemmell C.G., Lang S. (2008) Biofilm formation by Scottish clinical isolates of Staphylococcus aureus. J Med Microbiol. 8:1018-23.

21. Renuka J., Reddy K.I., Srihari K., Jeankumar V.U., Shravan M., Sridevi J.P., Yogeeswari P., Babu K.S., Sriram D. (2014) Design, synthesis, biological evaluation of substituted benzofurans as DNA gyraseB inhibitors of Mycobacterium tuberculosis. Bioorg Med Chem. 17:4924-34.

22. Jeankumar V.U., Renuka J., Kotagiri S., Saxena S., Kakan S.S., Sridevi J.P., Yellanki S., Kulkarni P., Yogeeswari P., Sriram D. (2014) gyrase ATPase domain as an

antitubercular drug discovery platform: structure-based design and lead optimization of nitrothiazolyl carboxamide analogues. ChemMedChem. 8:1850-9.

23. Alexandrou A.J.; Duncan R.S.; Sullivan A.; Hancox J.C.; Leishman D.J.; Witchel H.J.; Leaney J.L. (2006) Mechanism of hERG K+ channel blockade by the fluoroquinolone antibiotic moxifloxacin. Br. J. Pharmacol. 8:905-16.

24. Chen, X.; Cass, J. D.; Bradley, J. A.; Dahm, C. M.; Sun, Z.; Kadyszewski, E.; Engwall, M. J.; Zhou, J. Br. J. Pharmacol. 2005, 146, 792.

25. Milan D.J.; Peterson T.A.; Ruskin J.N.; Peterson R.T.; MacRae C.A. (2003) Drugs that induce repolarization abnormalities cause bradycardia in zebrafish. Circulation. 10:1355-8.

26. Mittelstadt S.; Hemenway C.; Craig M.; Hove J. (2008) Evaluation of zebrafish embryos as a model for assessing inhibition of hERG. J. Pharmacol. Toxicol. Methods. 2:100-5

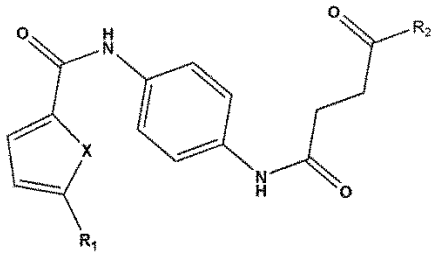
27. Brvar M.; Perdih A.; Renko M.; Anderluh G.; Turk D. A.; Solmajer T. (2012) Structure based discovery of substituted 4,5'-bithiazoles as novel DNA gyrase inhibitors. J. Med. Chem. 55, 6413.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1: Methodology used for docking, spectral details of all the synthesised compounds and the protocol used for biological evaluation.

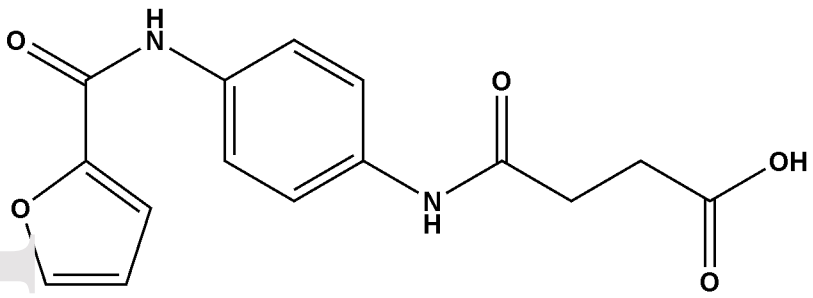
Table1. Biological activities of furan/pyrrole/thiophene-2-carboxamide derivatives

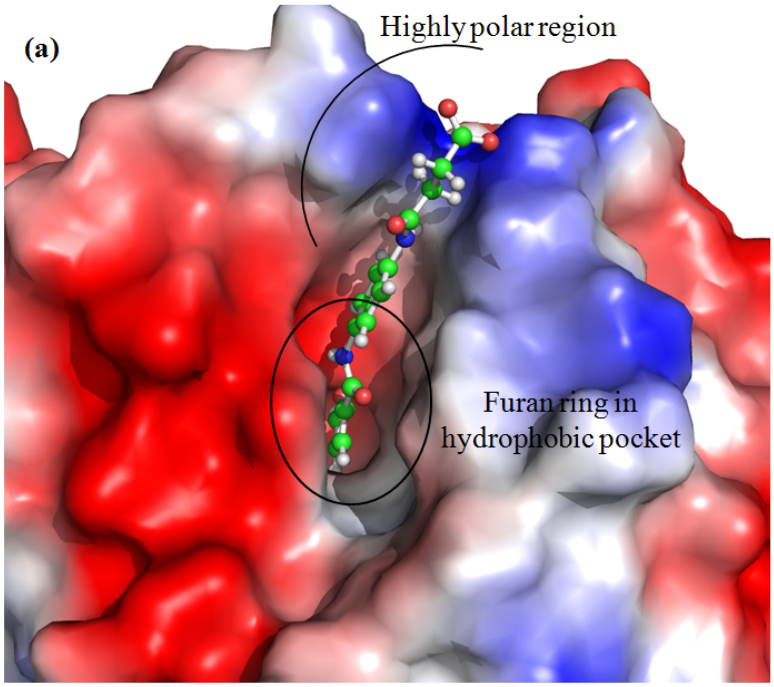


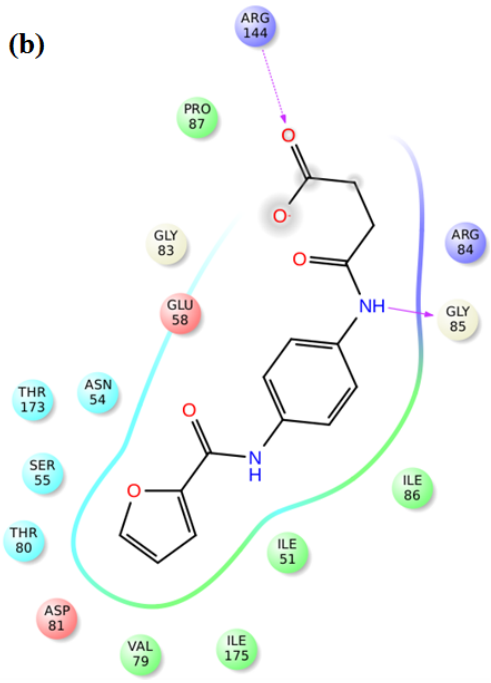
Comp	R_1	R_2	X	GyraseB assay(IC_{50})[a] (µM)	Supercoiling assay(IC_{50})[b] (µM)	MIC ATCC (µM)[c]	MRSA MIC (µM)[d]	Biofilm Inhibi. (IC_{50})[e]	Cytotoxicity (% inhibi.)[f]
5 (Lead)	H	-OH	O	12.88±1.39	3.12±1.6	79.03	79.03	79.03	15.95
6	CH_3	OCH3	O	27.63±1.09	26.15±0.3	75.67	75.67	75.67	21.22
7	NO_2	OCH3	O	22.64±0.66	8.41±0.22	17.29	34.58	34.58	19.88
8	H	OCH3	S	37.63±0.91	11.2±0.69	37.60	75.2	37.60	16.64
9	CH_3	OCH3	S	37.52±1.22	22.6±0.91	72.17	72.17	72.1	17.34
10	NO_2	OCH3	S	12.49±0.58	6.12±0.49	16.56	16.56	33.12	17.82
11	-H	$0CH_3$	THF	11.06±0.92	7.5±0.39	40.89	40.89	81.78	15.5
12	-H	OCH3	N	24.86±0.65	13.7±0.77	39.02	78.04	39.02	12.54
13	H	OCH3	O	45.42±2.54	28.18±0.6	79.03	158.06	79.03	17.66
14	CH_3	-OH	O	10.33±0.51	6.12±0.7	39.51	79.02	39.51	18.33
15	NO_2	-OH	O	28.94±0.83	25.8±0.35	35.99	71.98	35.99	13.26
16	H	-OH	S	21.99±0.74	12.5±0.51	39.26	78.52	39.26	24.62
17	CH_3	-OH	S	24.66±0.82	12.5±0.83	39.26	78.52	39.26	18.35
18	NO_2	-OH	S	29.49±0.81	26.8±1.25	68.81	68.81	39.26	18.55
19	-H	-OH	N	42.45±0.88	13.7±0.45	81.61	81.61	81.61	15.22
20	-H	-OH	THF	11.69±0.5	6.12±0.28	39.02	78.04	39.02	17.06
21	H	$-NH-NH_2$	O	39.41±0.97	23.9±0.4	79.03	79.03	79.03	14.21

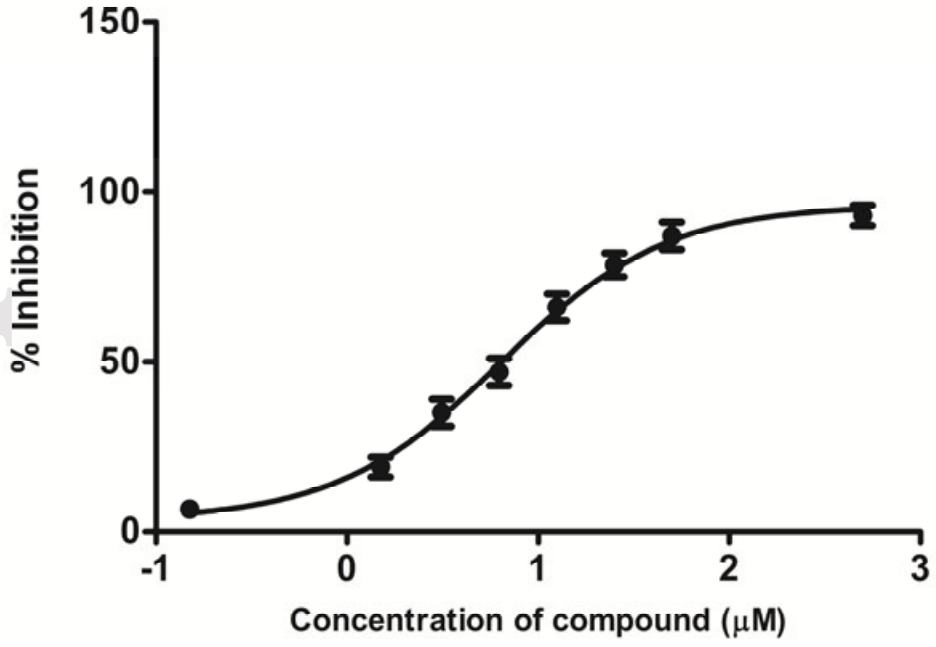
22	CH_3	-NH-NH_2	O	5.35±0.44	2.79±0.59	18.92	18.92	18.92	9.35
23	NO_2	-NH-NH_2	O	35.49±1.54	25.1±0.44	34.59	34.59	34.59	22.53
24	H	-NH-NH_2	S	32.56±2.41	28.1±0.67	75.23	75.23	75.23	10.22
25	CH_3	-NH-NH_2	S	9.71±0.69	6.12±0.22	18.04	36.08	36.08	19.31
26	NO_2	-NH-NH_2	S	26.44±0.77	19.3±0.88	68.05	68.05	68.05	13.54
27	-H	-NH-NH_2	N	54.87±0.84	43.1±0.68	78.03	156.06	156.06	17.33
28	-H	NH-NH_2	THF	41.2±0.83	23.1±0.45	78.03	156.06	78.03	15.4
Novobiocin				0.125 ± 0.24	0.03 ± 0.01	0.25	ND	ND	11.4
Moxifloxacin				0.81±0.15	ND	ND	ND	ND	ND

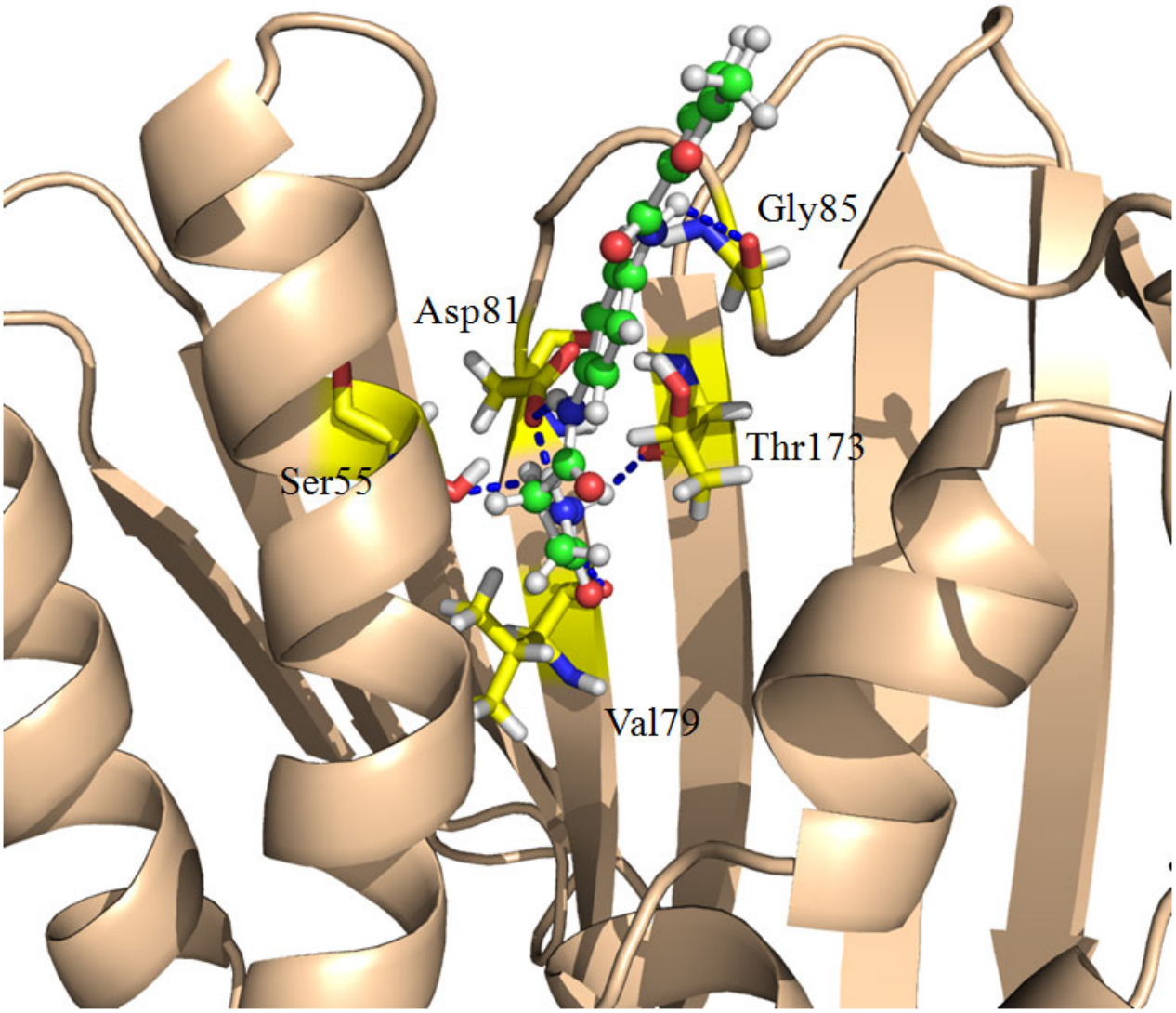
ND, indicates not determined.
[a] *S. aureus* gyraseB ATPase activity.
[b] *S. aureus* DNA gyrase supercoiling activity.
[c] *In vitro* (MTCC 3160) activity.
[d] *In vitro* (MTCC 96) MRSA MIC activity.
[e] Biofilm inhibition of MTCC 96.
[f] At 100 µM against RAW 264.7 cells.

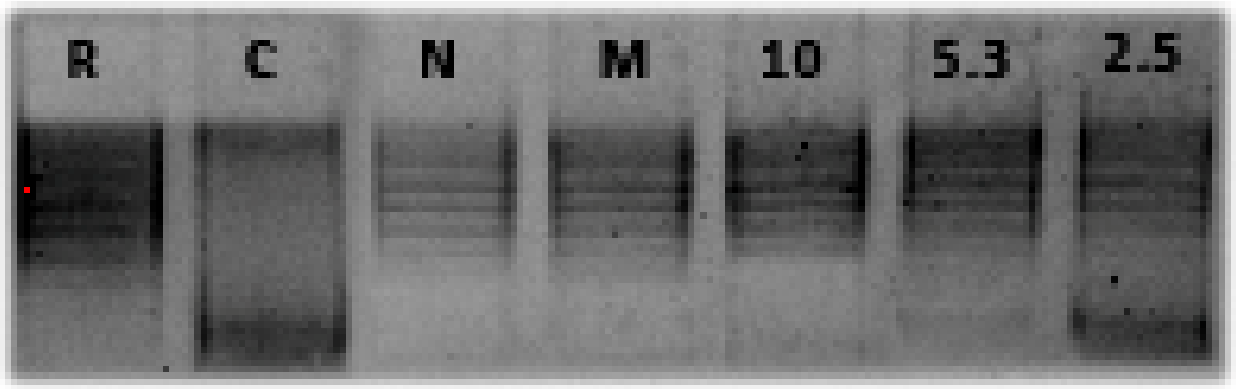


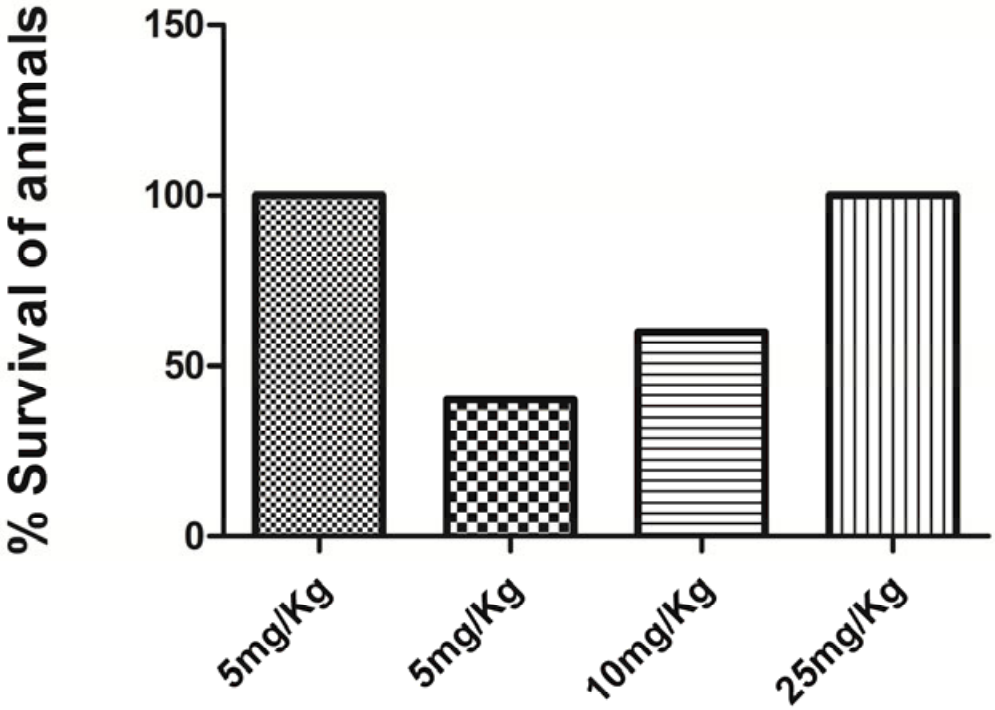


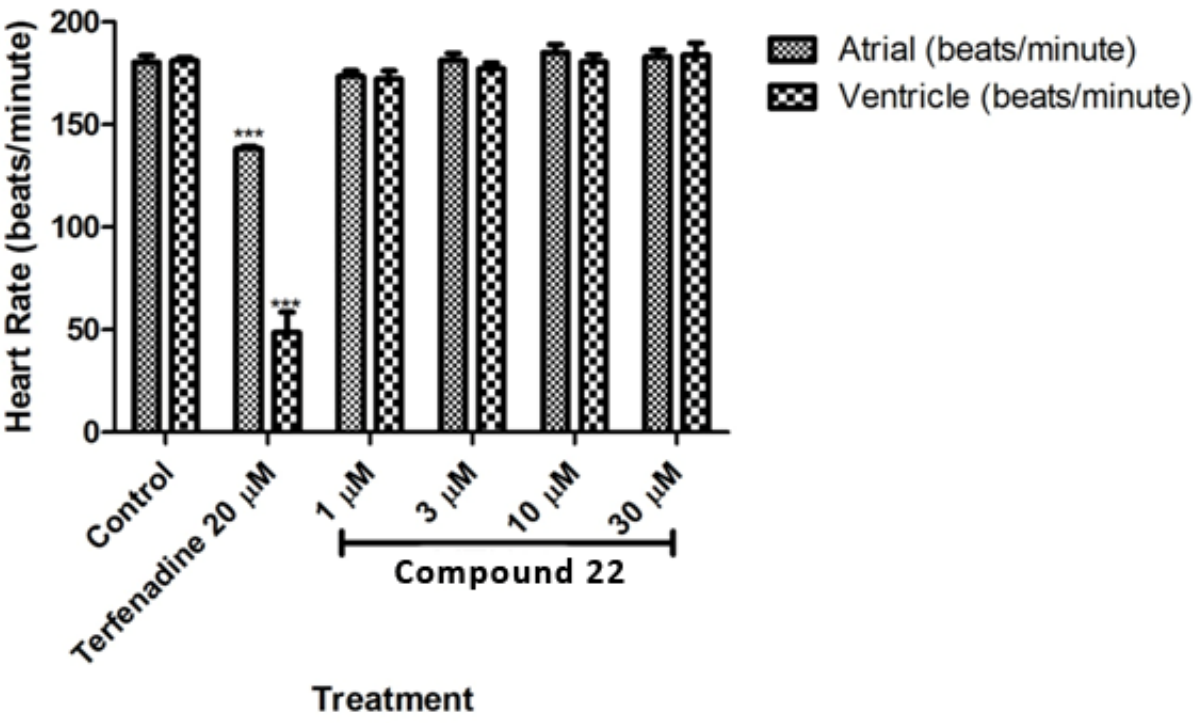


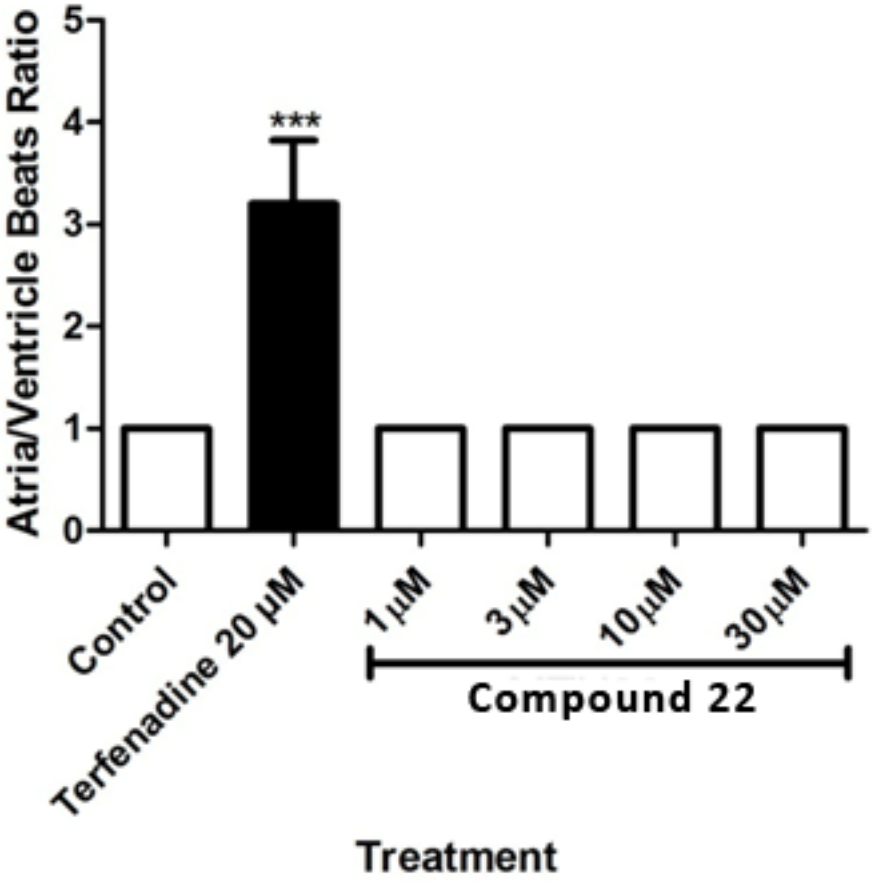


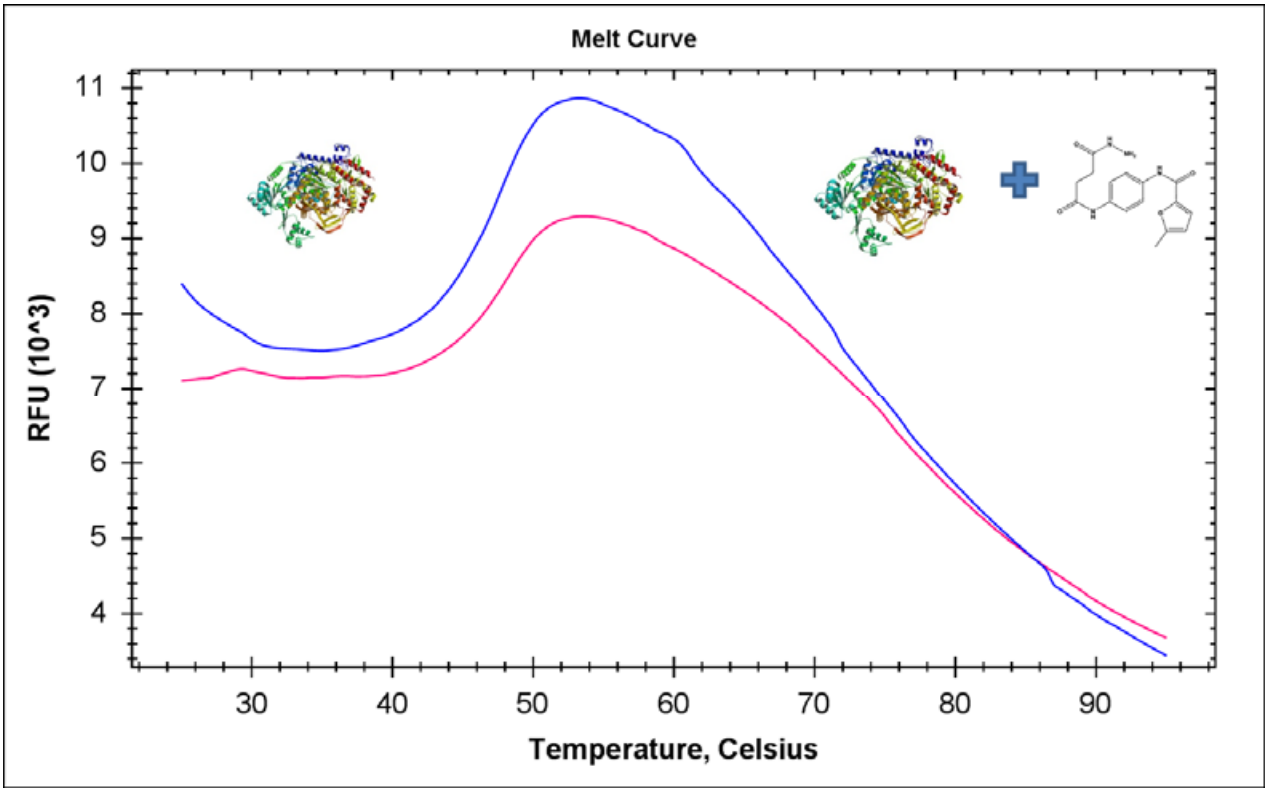


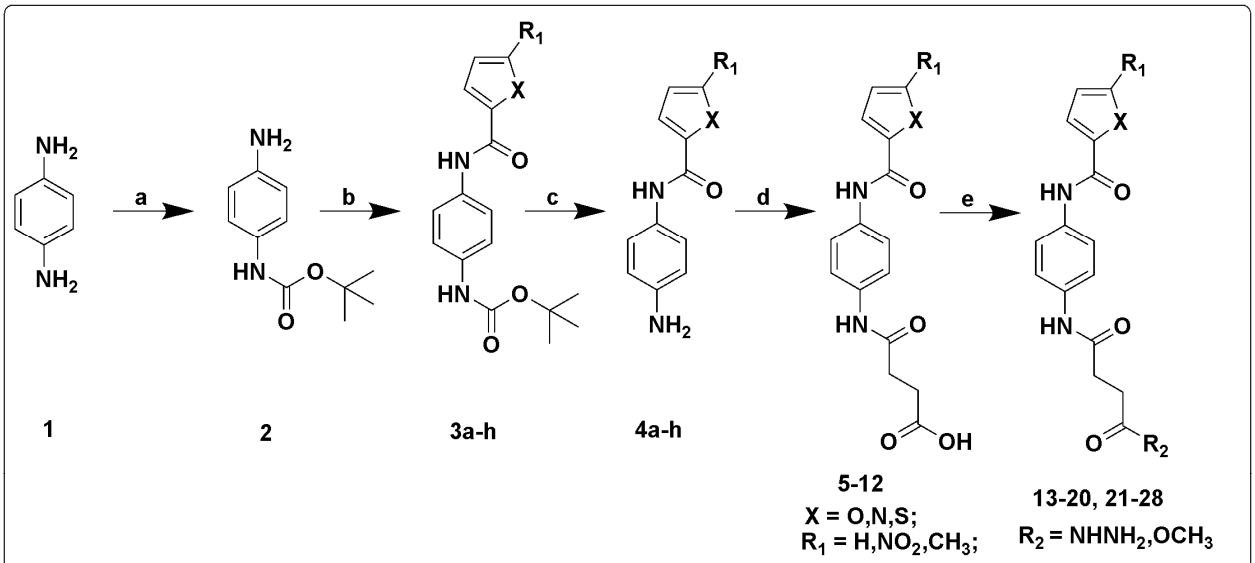












Reagents and conditions: a. $(Boc)_2O$, Et_3N, DMF, 6h, rt; b. corresponding acid chloride, Et_3N, DCM, 0-rt, 3h; c. TFA, DCM, 0-rt, 4h; d. anhydrous THF, 4 Å molecular sieves, succinic anhydride, 165 °C, microwave, 0.5h; e. 1. H_2SO_4 (cat), Methanol, reflux, 3h; 2. Hydrazine hydrate/ethanol, reflux, 4h.