Supporting information

Na$_2$S promoted reduction of azides in water: Synthesis of pyrazolopyridines in one pot and evaluation of antimicrobial activity

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Biological evaluation

Antimicrobial activity

The antimicrobial activity of the derivatives was tested based on well diffusion method\(^1\) against different pathogenic reference strains procured from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded with 0.1 ml of previously prepared microbial suspensions individually containing \(1.5 \times 10^8\) cfu ml\(^{-1}\) (equal to 0.5 McFarland) on the surface of Muller-Hinton agar Petri plates. Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized derivatives were added at a dose range of 150 - 1.17 µg well\(^{-1}\) in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of ciprofloxacin and miconazole at a dose range of 150 - 0.58 µg well\(^{-1}\) and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 37 °C for bacterial strains and 30 °C for *Candida albicans*. The well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration. All experiments were carried out in duplicates and mean values are represented.

Minimum bactericidal concentration (MBC) assay

Bactericidal assay\(^2\) (NCCLS, 2000) was performed in sterile 2.0 ml microfuge tubes against a panel of pathogenic bacterial strains, including *Micrococcus luteus* MTCC 2470, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, and *Klebsiella planticola* MTCC 530 which were cultured overnight in Mueller Hinton broth. Serial dilutions of test compounds were prepared in Mueller Hinton broth with different concentrations ranging from 0 to 250 µg mL\(^{-1}\). To the test compounds, 100 µL of overnight cultured bacterial suspensions were added to reach a final concentration of \(1.5 \times 10^8\) cfu mL\(^{-1}\) (equal to 0.5 McFarland) and incubated at 37 °C for 24 h. After 24 h of incubation, the minimum bactericidal concentration (MBC) was determined by sampling 10 µL of suspension from the tubes onto Mueller Hinton agar plates and were incubated for 24 h at 37 °C to observe the growth of test organisms. MBC is the lowest concentration of compound required to kill a particular bacterium. All the experiments were carried in duplicates.

References:

$^1$H-NMR and $^{13}$C-NMR spectra of compound 2a
¹H-NMR and ¹³C-NMR spectra of compound 2b
$^1$H-NMR and $^{13}$C-NMR spectra of compound 2c
$^1$H-NMR and $^{13}$C-NMR spectra of compound 2d
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$^1$H-NMR and $^{13}$C-NMR spectra of compound 2g
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$^1$H-NMR, $^{19}$F-NMR and $^{13}$C-NMR spectra of compound 4a
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4b
$^{1}$H-NMR and $^{13}$C-NMR spectra of compound 4c
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$^1$H-NMR, $^{19}$F-NMR and $^{13}$C-NMR spectra of compound 4e
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4f
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$^1$H-NMR and $^{13}$C-NMR spectra of compound 4i
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4j
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4k
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4l
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4m
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4n
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4o
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4p
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4q
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4r
$^1$H-NMR and $^{13}$C-NMR spectra of compound 4s
$^{1}$H-NMR and $^{13}$C-NMR spectra of compound 4t