Antibiotics are commonly used to treat microbial infections. Due to misuse or large-scale use of antibiotics, many pathogens have gained resistance which makes antibiotic treatments ineffective. The discovery that many bacteria use quorum sensing (QS) to regulate their virulence factor and pathogenicity production makes the QS system an attractive target for antimicrobial therapy. A series of 1,3-benzoxazol-2(3H)-one derivatives were designed and synthesized as QS inhibitors (QSIs) and tested for their QS inhibitory activities. In vitro quorum sensing inhibitor screen (QSI) assay indicated that the 1,3-benzoxazol-2(3H)-one (compound 1), 5-chloro-1,3-benzoxazol-2(3H)-one (compound 6), 6-methyl-1,3-benzoxazol-2(3H)-one (compound 11), and 5-methyl-1,3-benzoxazol-2(3H)-one (compound 16), inhibit QS system in quorum sensing selector (QSI)1 strain. These 4 QSIs also significantly reduced elastase production, biofilm formation and swarming motility of Pseudomonas aeruginosa PA01 strain. These results suggest that compound 1, 6, 11 and 16 may provide a starting point for the design and development of new anti-pathogenic drugs that restrict virulence of P. aeruginosa and possibly other clinically important human pathogens. In addition, these QSI molecules could potentially be used in combination with conventional antibiotics to increase the efficiency of disease control and to extend the life span of established antimicrobials.