Electronic Supplementary Information

**Design, synthesis, and evaluation of curcumin analogues as potential inhibitors of bacterial sialidase**

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2. Preparation of enzyme

```plaintext
1 gaaggagcgcttaacagagaaaaacgacattcgaagacggttaacggttaaacc
E G A A L T E K T D I F E S G R N G K P
61 aataaagatggaatcaagagttatctgatattcagaacactctcaagagacataaaggaact
N K D G I K S Y R I P A L L K T D K G T
121 ttgatgcaggtgcagatgaacgcgcgtctccattcagtgactgaggtgtatcggatatg
L I A G A D E R L H S D W G D I G M
181 gtcatcagcagctgtgaagataatggtaaaactttgggtgaccgagtaaccattacacac
V I R R S E N G K T W G D R V T I T N
241 ttacgtgacaatccaaacgctctctcagctggttgatcggtatatcgatatg
L R D N P K A S D P S I G S P V N I D M
301 gtgttggttcaagatgctctctcaacaaaaggaagaagctctcataaatgcgtgga
V L V Q D P E T K R I F S I Y D M F E P E
361 gggagaaggagtctttggaatgtcttcacaaaaaagaagaagctctcataaatgcgtgga
G K G I F G M S S Q K E E A Y K K I D G
421 aaaaactctcaatcgtctctgtgaagagataatggtaaaactttgggtgaccgagtaaccattacacac
K T Y Q I L Y R E G K G A Y T I R E N
481 ggtagctgctctataccagcttgtggtacgcgacagatctgcgtgttgtagatcctgtt
G T V Y T P K A T D Y R V V V D P V
541 aaacccagctctagcgcacacaggggtatataacccaaatgagtcataatggcagatc
K P A Y S D K G D L Y K G N Q L L G N I
601 taccctcagaccaacaaacaaactctctctttagaattgacgatagctatcttagatg
Y F T T N K T S P F R I A K D S Y L W M
661 ttctagctgatgacgacgaggaagcatactgcacgcctcaagatattacccgatggtc
S Y S D D G K T W S A P Q D I T P M V
721 aacaacctatgaagatctttggtagatgtctgctgaacagaaatggtacttccgaaat
K A D W M K F L G V G P G T G I V L R N
781 gggcctcacaagggacggatatttgataccggtttatatagactaataatgatattacactta
G P H K G R I L I P V Y T T N N V S H L
841 aatggtctgcaatctctctgtatcattatcagatgatcatgaaaaacactttggcattgct
N G S Q S S R I I Y S D D H G K T W H A
901 gagaagccggtacaagataacccggtagagacggtcacaagatcactctctcagtg
G E A V N D N R Q V D G Q K I H S S T M
961 aacaataagctgacgcaaaaattacaagataacccggttagagacggtcacaagatcactctctcagtg
N N R A Q N T E S T V V Q L N N G D V
1021 aacaacctagacgtgttcatgagctttgactttgagatctcaggctgctcaagaaatgatataagagacgagga
K L F M R G L T G D L Q V A T S K D G G
1081 ggtagcttggagaaggatatcaacagctttcaccaggttaaagatgctatgttctaatag
V T W E K D I K R Y P Q V K D V V Y V Q M
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Figure S28. Nucleotide sequence and protein sequence of synthesized *S. pneumoniae* neuraminidase A (NanA)

Figure S29. Expression and purification of *S. pneumoniae* NanA.
Figure S30. Michaelis-Menten plots (A) and Lineweaver-Burk plot (B) of *S. pneumoniae* NanA $K_m$ values. The reaction was performed at various substrate concentrations to obtain enzyme $K_m$ values. SigmaPlot was used to fit the kinetic data using Michaelis-Menten and Lineweaver-Burk double reciprocal plots.
**Figure S31.** Graphical determination of the inhibition type for compounds 4a, 4e, 5q and 5e. Lineweaver-Burk (A-D) and Dixon (E-H) plots for the inhibitory activity of compounds 4a, 4e, 5q and 5e, respectively, against *S. pneumoniae* NanA hydrolysis activity in the presence of different substrate concentrations.
Figure S31. Graphical determination of the inhibition type for compounds 4a, 4e, 5q and 5e. Lineweaver-Burk (A-D) and Dixon (E-H) plots for the inhibitory activity of compounds 4a, 4e, 5q and 5e, respectively, against *S. pneumoniae* NanA hydrolysis activity in the presence of different substrate concentrations (continued).