Supplementary Materials

Evaluation of synergistic effect of heteroaryl-ethylene molecules in combination with antibiotics: a preliminary study on control strains.

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**Table S1**. Structures of the 13 compounds added to the QSAR model for antimicrobial activity against S. aureus ATCC29213 [1]

|  |  |  |  |
| --- | --- | --- | --- |
| **Molecules & Smiles string** | **ID VS+** | **MIC (µg/mL)** | **[Ref]** |
| CN(C)c1ccc(\C=C\c2ccc3ccccc3[n+]2C)cc1 | **PB1** | 4 |  |
| Immagine che contiene oscurità, nero, schermata, notte  Descrizione generata automaticamenteCCCCN(CCCC)c1ccc(s1)c2ccc(\C=C\c3ccc4ccccc4[n+]3C)s2 | **PB2** | 4 |
| C[n+]1c(\C=C\c2ccc(s2)c3ccccc3)ccc4ccccc14 | **PB3** | 4 |
| CN(C)c1ccc(\C=C\c2cccc(\C=C\c3ccc(cc3)N(C)C)[n+]2C)cc1 | **PB4** | 0.25 |
| Clc1ccc(Cl)c(c1)c2oc(\C=C\c3cc[n+](cc3)c4ncccn4)cc2 | **PB5** | 1 |
| C[n+]1ccc(\C=C\c2oc(cc2)c3cc(Cl)ccc3Cl)cc1 | **PB6** | 4 |
| CC[n+]1ccc2\C(=C\c3oc(cc3)c4ccc(Cl)cc4)\CCCc2c1 | **PB7** | 4 |
| CCCCN(CCCC)c1ccc(s1)c2ccc(\C=C\3/CCCc4c[n+](CC)ccc34)s2 | **PB8** | 2 | [1] |

**Table S1.** Continue

|  |  |  |  |
| --- | --- | --- | --- |
| CC[n+]1ccc2\C(=C\c3ccccc3O)\CCCc2c1 | **BCNAc1** | >128 | [1] |
| CC[n+]1ccc2\C(=C\c3ccc(cc3)N(C)C)\CCCc2c1 | **BCM3** | 64 |
| C[n+]1c(\C=C\c2cc(Cl)ccc2O)cccc1\C=C\c3cc(Cl)ccc3O | **BCM12** | >128 |
| CC[n+]1c(\C=C\c2cn(CC)c3ccccc23)ccc4ccccc14 | **SQL** | 4 | [2] |
| CC[n+]1c(\C=C\c2cn(CC)c3ccccc23)sc4ccccc14 | **SBT** | 1 |

\*Unpublished results



**Figure S1.** Score plot of Principal Component Analysis (PCA) at the third component (PC1 *vs* PC2 *vs* PC3) for the 49 Heteroaromatic compounds tested for antimicrobial activity against *S. aureus* ATCC29213. Compounds are color-coded by their activity values, using a scale from red (actives) to blue (inactives), according to the experimental MIC values.



**Figure S2.** Plot of the coefficient of determination R2 and cross-validated coefficient of determination (leave-one-out, LOO) Q2 vs. the number of LVs of the PLS models for antimicrobial activity against S. aureus ATCC29213.



**Figure S3.** Plot of the Variable Influence on Projection VIP of the PLS models for antimicrobial activity against S. aureus ATCC29213.



**Figure S4.** Plot of the Weights for VS+ descriptors at the third latent variable (LV1 *vs* LV2 *vs* LV3) of the PLS models for antimicrobial activity against S. aureus ATCC29213. The yellow circle represents the dependent variable (MIC).

**Table S2.** Structures of the 38 heteroaryl ethylene compounds of the QSAR model for cytotoxic activity towards CaCo2 colon-rectal cancer cell line

|  |  |  |  |
| --- | --- | --- | --- |
| **Molecules & Smiles string** | **ID VS+** | **IC50 48h** | **Log(IC50) 48h** |
| C[n+]1c(\C=C\c2ccc(s2)c3ccc(s3)N4CCCCC4)ccc5ccccc15 | BC1 | 20 | 1.30 |
| N#CC(=Cc1ccc(s1)c2ccc(s2)N3CCCCC3)C#N | BC2 | 100 | 2 |
| CCOC(=O)\C(=C\c1ccc(s1)c2ccc(s2)N3CCCCC3)\C#N | BC3 | 250 | 2.40 |
| OC(=O)\C(=C\c1ccc(s1)c2ccc(s2)N3CCCCC3)\C#N | BC4 | 250 | 2.40 |
| C[n+]1ccccc1\C=C\c2ccc(cc2)c3cncnc3 | BC5 | 100 | 2 |
| C[n+]1c(\C=C\c2ccc(cc2)c3cncnc3)ccc4ccccc14 | BC6 | 25 | 1.5 |
| CCCCN(CCCC)c1ccc(s1)c2ccc(\C=C\c3ccc(c[n+]3C)C(=O)O)s2 | BCG3 | 8 | 0.90 |
| CC[n+]1ccc2\C(=C\c3ccc(cc3)N(C)C)\CCCc2c1 | BCM3 | 1.35 | 0.13 |
| COc1ccc(\C=C\c2cccc(\C=C\c3ccc(OC)cc3)[n+]2C)cc1 | BCM6 | 12.34 | 1.09 |

Table S2. Continue

|  |  |  |  |
| --- | --- | --- | --- |
| C[n+]1c(\C=C\c2cc(Cl)ccc2O)cccc1\C=C\c3cc(Cl)ccc3O | BCM12 | 250 | 2.40 |
| COc1cc(\C=C\c2cccc[n+]2C)cc(OC)c1OC | GC VI 2 | 250 | 2.40 |
| C[n+]1ccccc1\C=C\c2ccc3ccc4CC=CC5C=Cc2c3c45 | GC VI 3 | 0.9 | -0.0458 |
| Immagine che contiene oscurità, schermata, nero, spazio  Descrizione generata automaticamenteC[n+]1c(\C=C\c2ccc(cc2)N(c3ccccc3)c4ccccc4)ccc5ccccc15 | GC VI 14 | 4 | 0.602 |
| CN(C)c1ccc(\C=C\c2cc[n+](C)cc2)cc1 | GC VI 17 | 8 | 0.903 |
| CN(C)c1ccc(\C=C(/C#N)\c2cccc[n+]2C)cc1 | GC VI 26 | 100 | 2 |
| CN(C)c1ccc(\C=C\C=C\c2cc[n+](C)cc2)cc1 | GC VI 45 | 16 | 1.20 |
| Immagine che contiene schermata, oscurità, nero, spazio  Descrizione generata automaticamenteC[n+]1c(\C=C\c2ccnc(c2)c3cc(\C=C\c4ccc5ccccc5[n+]4C)ccn3)ccc6ccccc16 | GC VI 71 | 51.2 | 1.71 |
| Immagine che contiene schermata, oscurità, nero  Descrizione generata automaticamenteCCOC(=O)C[n+]1ccc(\C=C\c2ccc(C(C#N)C#N)n2C)cc1 | GC VII 13 | 100 | 2 |

Table S2. Continue

|  |  |  |  |
| --- | --- | --- | --- |
| Immagine che contiene schermata, oscurità, nero  Descrizione generata automaticamenteCCOC(=O)C(C#N)c1ccc(\C=C\c2cc[n+](C)cc2)n1C | GC VII 16 | 70 | 1.85 |
| Immagine che contiene schermata, oscurità, nero  Descrizione generata automaticamenteCn1c(\C=C\C2=C(C#N)C(=C(C#N)C#N)OC2(C)C)ccc1\C=C\c3cc[n+](CC(=O)OC(C)(C)C)cc3 | GC VII 41 | 100 | 2 |
| C[n+]1ccccc1\C=C\c2ccc3ccccc3c2 | GC VII 46 | 5.5 | 0.740 |
| C[n+]1ccccc1\C=C\c2ccc(cc2)N(c3ccccc3)c4ccccc4 | GC VII 47 | 1 | 0 |
| Cn1c(\C=C\C2=CC=C3C=CC=CC3=[N]2C)ccc1\C=C\c4ccncc4 | GF I 10 | 20 | 1.30 |
| Immagine che contiene oscurità, schermata, nero  Descrizione generata automaticamenteCN(C)c1ccc(\C=C\C=C\c2cccc[n+]2C)cc1 | GF I 25 | 10 | 1 |
| Immagine che contiene schermata, oscurità, nero, spazio  Descrizione generata automaticamenteC[n+]1ccc(\C=C\c2ccc(\C=C\c3ccncc3)n2C)cc1 | GF I 30 | 50 | 1.70 |
| C[n+]1ccc(\C=C\c2ccc(C=C(C#N)C#N)n2C)cc1 | GF I 31 | 100 | 2 |
| C[n+]1ccc(\C=C\c2ccc(\C=C\C3=C(C#N)C(=C(C#N)C#N)OC3(C)C)n2C)cc1 | GF I 32 | 32 | 1.51 |

Table S2. Continue

|  |  |  |  |
| --- | --- | --- | --- |
| CN(C)c1ccc(\C=C\c2ccc3ccccc3[n+]2C)cc1 | PB1 | 0.35 | -0.456 |
| Immagine che contiene oscurità, nero, schermata, notte  Descrizione generata automaticamenteCCCCN(CCCC)c1ccc(s1)c2ccc(\C=C\c3ccc4ccccc4[n+]3C)s2 | PB2 | 0.18 | -0.745 |
| C[n+]1c(\C=C\c2ccc(s2)c3ccccc3)ccc4ccccc14 | PB3 | 1.3 | 0.114 |
| CN(C)c1ccc(\C=C\c2cccc(\C=C\c3ccc(cc3)N(C)C)[n+]2C)cc1 | PB4 | 0.33 | -0.481 |
| Clc1ccc(Cl)c(c1)c2oc(\C=C\c3cc[n+](cc3)c4ncccn4)cc2 | PB5 | 0.95 | 0.246 |
| C[n+]1ccc(\C=C\c2oc(cc2)c3cc(Cl)ccc3Cl)cc1 | PB6 | 3.22 | 0.508 |
| CC[n+]1ccc2\C(=C\c3oc(cc3)c4ccc(Cl)cc4)\CCCc2c1 | PB7 | 1.36 | 0.134 |
| CCCCN(CCCC)c1ccc(s1)c2ccc(\C=C\3/CCCc4c[n+](CC)ccc34)s2 | PB8 | 5.04 | 0.702 |
| COc1ccc(\C=C\c2ccc3ccc4ccc(\C=C\c5ccc(OC)cc5)nc4c3n2)cc1 | PB9 | 250 | 2.40 |

Table S2. Continue

|  |  |  |  |
| --- | --- | --- | --- |
| CN(C)c1ccc(\C=C\c2ccc3ccc4ccc(\C=C\c5ccc(cc5)N(C)C)nc4c3n2)cc1 | PB10 | 10.6 | 1.03 |
| C(=C\c1ccc2ccc3ccc(\C=C\c4ccc(cc4)N(c5ccccc5)c6ccccc6)nc3c2n1)/c7ccc(cc7)N(c8ccccc8)c9ccccc9 | PB11 | 11.5 | 1.06 |



**Figure S5.** Score plot of Principal Component Analysis (PCA) at the third component (PC1 *vs* PC2 *vs* PC3) for the 38 Heteroaromatic compounds tested for cytotoxic activity towards CaCo2 colon-rectal cancer cell line. Compounds are color-coded by their activity values, using a scale from red (actives) to blue (inactives), according to the experimental MIC values.



**Figure S6.** Plot of the coefficient of determination R2 and cross-validated coefficient of determination (leave-one-out, LOO) Q2 vs. the number of LVs of the PLS models for cytotoxic activity towards CaCo2 colon-rectal cancer cell line.



**Figure S7.** Plot of the Variable Influence on Projection VIP of the PLS models for cytotoxic activity towards CaCo2 colon-rectal cancer cell line.



**Figure S8.** Plot of the Weights for VS+ descriptors at the third latent variable (LV1 *vs* LV2 *vs* LV3) of the PLS models for cytotoxic activity towards CaCo2 colon-rectal cancer cell line. The yellow circle represents the dependent variable the logarithm of the IC50.

**Table S3.** Dunnett’s multiple comparisons test performed for all combinations.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Dunnett's multiple comparisons test** | **Mean Diff** | **95% CI of diff** | **Below threshold?** | **Summary** | **Adjusted P Value** |
| **ANTIBIOTICS 24h** |
| Untreated vs. T0 | 48,44 | 29,50 to 67,39 | Yes | \*\*\*\* | <0,0001 |
| Untreated vs. Linezolid MIC | -3,090 | -36,67 to 30,49 | No | ns | 0,9997 |
| Untreated vs. Linezolid subMIC | 7,008 | -26,57 to 40,59 | No | ns | 0,9993 |
| Untreated vs. Gentamicin MIC | -7,797 | -41,38 to 25,78 | No | ns | 0,9992 |
| Untreated vs. Gentamicin subMIC | 18,12 | -15,46 to 51,70 | No | ns | 0,7346 |
| Untreated vs. Ampicillin MIC | 2,723 | -30,86 to 36,30 | No | ns | 0,9998 |
| Untreated vs. Ampicillin subMIC | -5,167 | -38,75 to 28,41 | No | ns | 0,9996 |
| Untreated vs. Erythromycin MIC | 2,213 | -31,37 to 35,79 | No | ns | 0,9998 |
| Untreated vs. Erythromycin subMIC | -9,070 | -42,65 to 24,51 | No | ns | 0,9963 |
| Untreated vs. Rifampicin MIC | 16,68 | -16,90 to 50,26 | No | ns | 0,8188 |
| Untreated vs. Rifampicin subMIC | 3,783 | -29,80 to 37,36 | No | ns | 0,9997 |
| **ANTIBIOTICS + PB4 0,2 µM 24h** |
| Untreated vs. T0 | 52,99 | 37,65 to 68,32 | Yes | \*\*\*\* | <0,0001 |
| Untreated vs. Linezolid MIC | 10,99 | -16,19 to 38,17 | No | ns | 0,9435 |
| Untreated vs. Linezolid subMIC | 22,91 | -4,265 to 50,09 | No | ns | 0,1618 |
| Untreated vs. Gentamicin MIC | 29,40 | 2,225 to 56,58 | Yes | \* | 0,0252 |
| Untreated vs. Gentamicin subMIC | 22,53 | -4,648 to 49,71 | No | ns | 0,1778 |
| Untreated vs. Ampicillin MIC | 21,13 | -6,045 to 48,31 | No | ns | 0,2471 |
| Untreated vs. Ampicillin subMIC | 19,39 | -7,785 to 46,57 | No | ns | 0,3570 |
| Untreated vs. Erythromycin MIC | 21,39 | -5,793 to 48,56 | No | ns | 0,2332 |
| Untreated vs. Erythromycin subMIC | 30,08 | 2,905 to 57,26 | Yes | \* | 0,0202 |
| Untreated vs. Rifampicin MIC | 28,09 | 0,9099 to 55,27 | Yes | \* | 0,0380 |
| Untreated vs. Rifampicin subMIC | 24,31 | -2,865 to 51,49 | No | ns | 0,1126 |
| **ANTIBIOTICS 48h** |
| Untreated vs. T0 | 136,6 | 104,1 to 169,1 | Yes | \*\*\*\* | <0,0001 |
| Untreated vs. Linezolid MIC | -14,37 | -71,94 to 43,20 | No | ns | 0,9968 |
| Untreated vs. Linezolid subMIC | -9,069 | -66,64 to 48,50 | No | ns | 0,9995 |
| Untreated vs. Gentamicin MIC | -8,939 | -66,51 to 48,63 | No | ns | 0,9995 |
| Untreated vs. Gentamicin subMIC | -33,12 | -90,69 to 24,45 | No | ns | 0,6570 |
| Untreated vs. Ampicillin MIC | -82,54 | -140,1 to -24,97 | Yes | \*\*\* | 0,0008 |
| Untreated vs. Ampicillin subMIC | -8,984 | -66,55 to 48,58 | No | ns | 0,9995 |
| Untreated vs. Erythromycin MIC | -30,40 | -87,97 to 27,16 | No | ns | 0,7585 |
| Untreated vs. Erythromycin subMIC | -42,07 | -99,64 to 15,50 | No | ns | 0,3247 |
| Untreated vs. Rifampicin MIC | -44,02 | -101,6 to 13,54 | No | ns | 0,2669 |
| Untreated vs. Rifampicin subMIC | -27,05 | -84,62 to 30,51 | No | ns | 0,8635 |
| **ANTIBIOTICS + PB4 0,2 µM 48h** |
| Untreated vs. T0 | 84,25 | 63,16 to 105,3 | Yes | \*\*\*\* | <0,0001 |
| Untreated vs. Linezolid MIC | 40,56 | 3,180 to 77,95 | Yes | \* | 0,0245 |
| Untreated vs. Linezolid subMIC | 55,66 | 18,28 to 93,05 | Yes | \*\*\* | 0,0005 |
| Untreated vs. Gentamicin MIC | 41,74 | 4,358 to 79,13 | Yes | \* | 0,0186 |
| Untreated vs. Gentamicin subMIC | 86,73 | 49,35 to 124,1 | Yes | \*\*\*\* | <0,0001 |
| Untreated vs. Ampicillin MIC | 58,50 | 21,12 to 95,89 | Yes | \*\*\* | 0,0002 |
| Untreated vs. Ampicillin subMIC | 49,22 | 11,83 to 86,60 | Yes | \*\* | 0,0028 |
| Untreated vs. Erythromycin MIC | 70,36 | 32,97 to 107,7 | Yes | \*\*\*\* | <0,0001 |
| Untreated vs. Erythromycin subMIC | 30,43 | -6,955 to 67,81 | No | ns | 0,1963 |
| Untreated vs. Rifampicin MIC | 27,48 | -9,900 to 64,87 | No | ns | 0,3169 |
| Untreated vs. Rifampicin subMIC | 22,49 | -14,89 to 59,88 | No | ns | 0,5974 |