ELECTRONIC SUPPLEMENTARY INFORMATION

Solid-state landscape and biopharmaceutical implications of novel metformin based-salts

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COMPLEMENTARY FIGURES AND TABLES

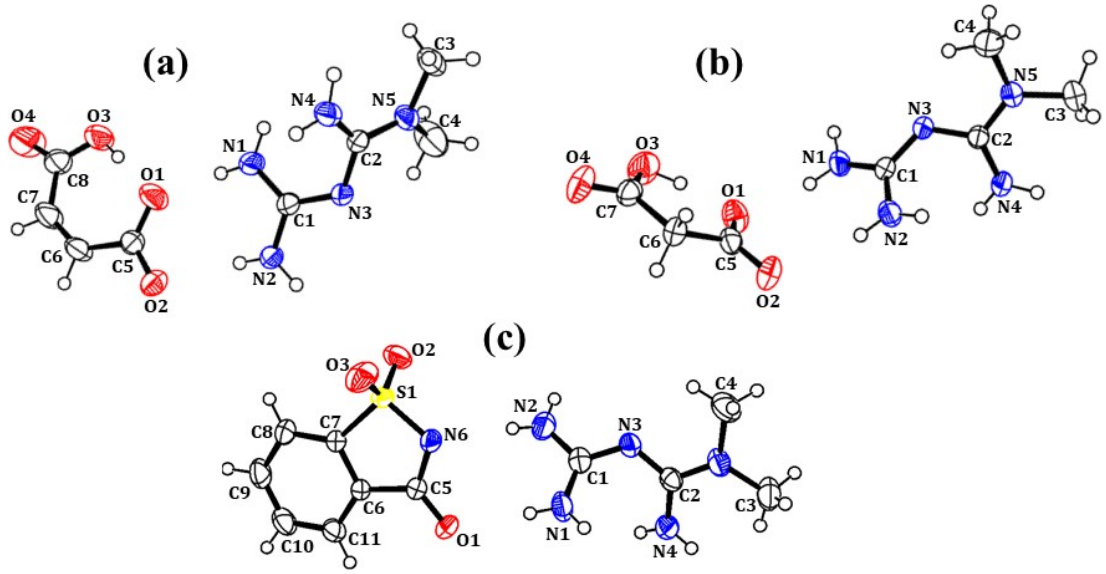


Figure S1. ORTEP type diagrams of the MET salts asymmetric units with 50% probability ellipsoids showing atomic numbering scheme: (a) metformin maleate (MET-MAL), (b) metformin malonate (MET-MLN), and (c) metformin saccharinate (MET-SAC). Hydrogen atoms are shown as spheres of arbitrary radii.

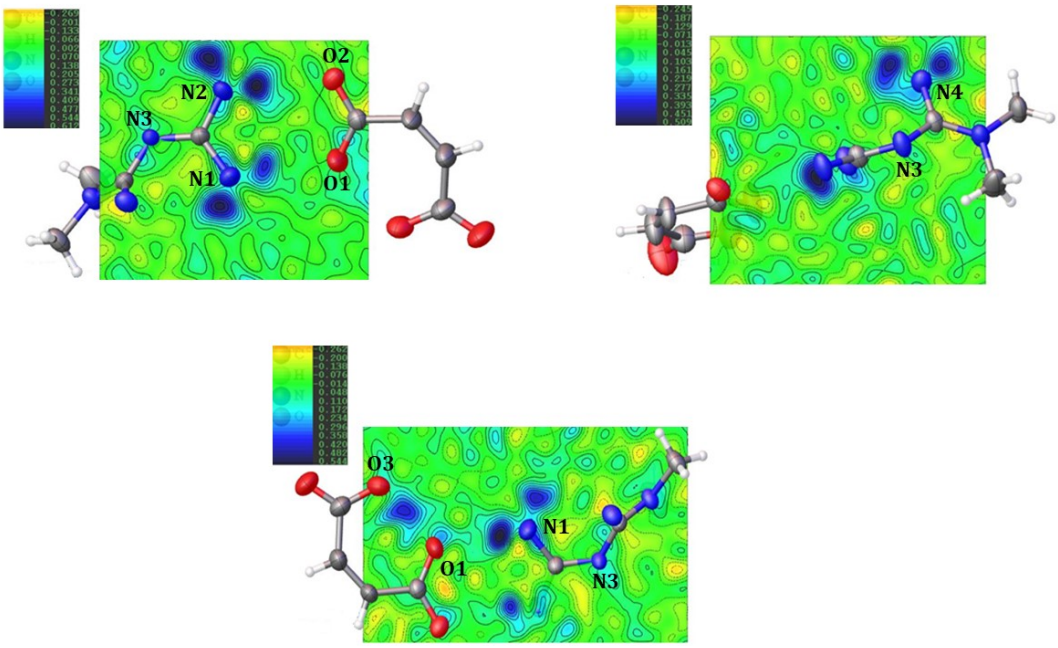


Figure S2. Fourier difference maps, under different perspectives, of both metformin cation (MET^{+}) and maleate anion (MAL^{-}) molecules from MET-MAL salt, showing in which O- and N-atoms the H-atoms are attached (blue spots).

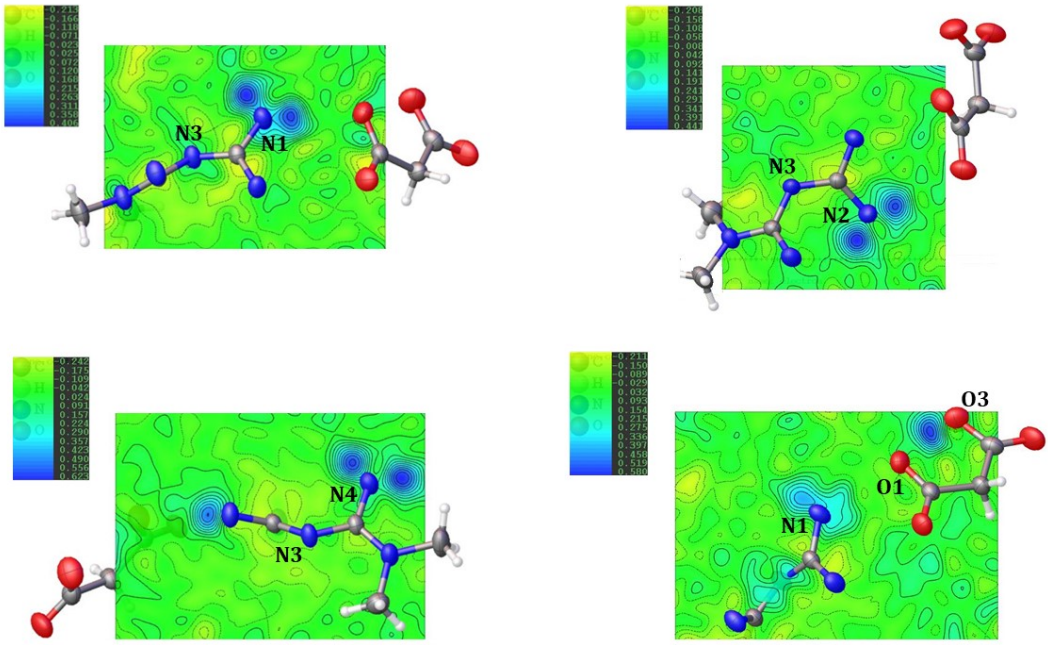


Figure S3. Fourier difference maps, under different perspectives, of both metformin cation (MET^{+}) and malonate anion (MLN^{-}) molecules from MET-MLN salt, showing in which O- and N-atoms the H-atoms are attached (blue spots).

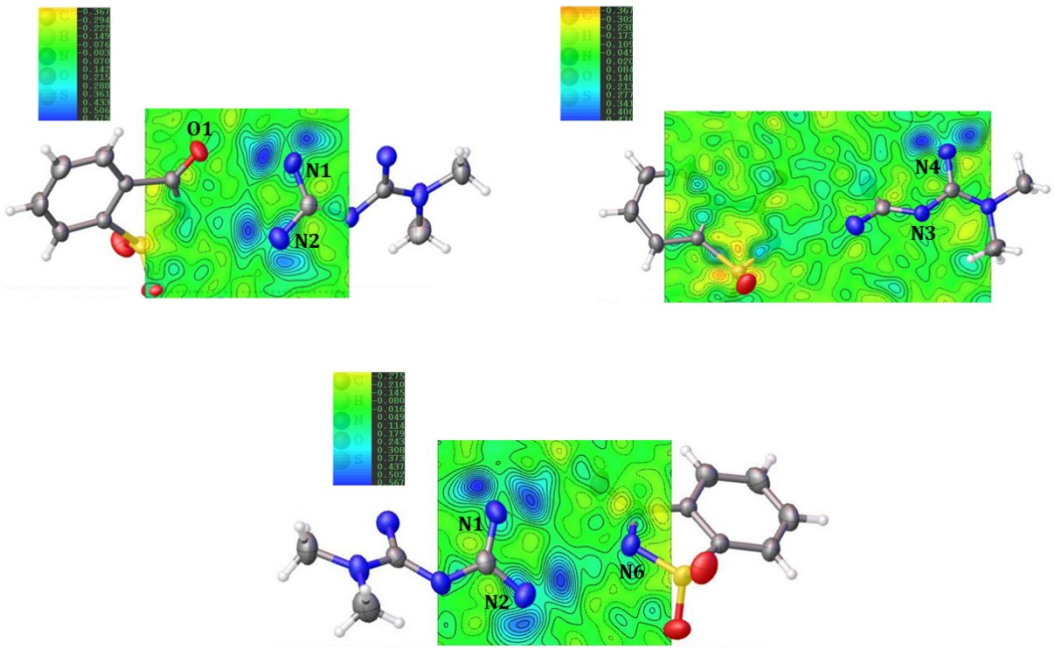


Figure S4. Fourier difference maps, under different perspectives, of both metformin cation (MET^{+}) and saccharinate anion (SAC^{-}) molecules from MET-SAC salt, showing in which O- and N-atoms the H-atoms are attached (blue spots).

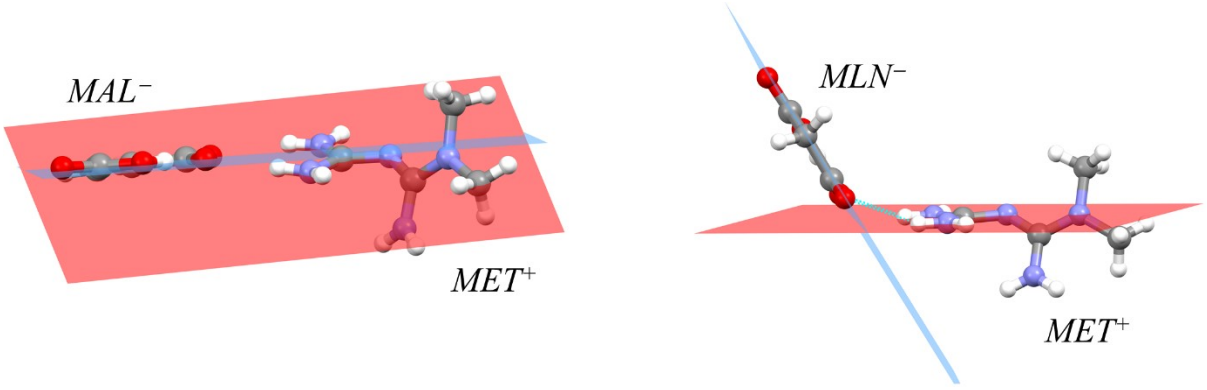
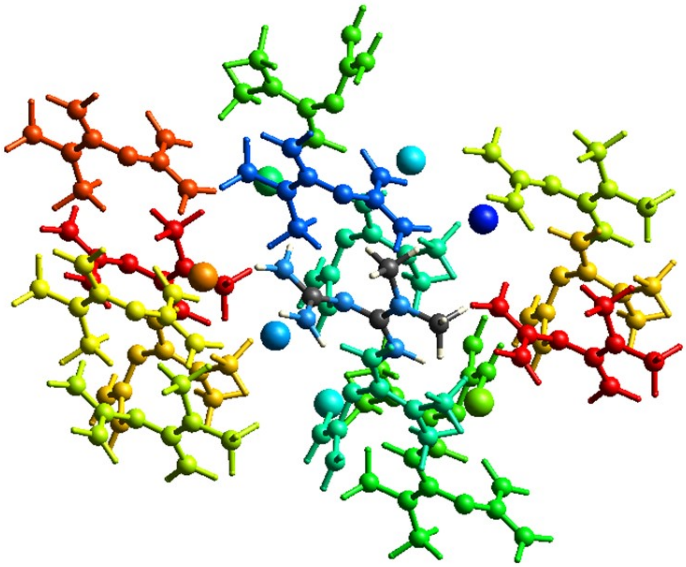
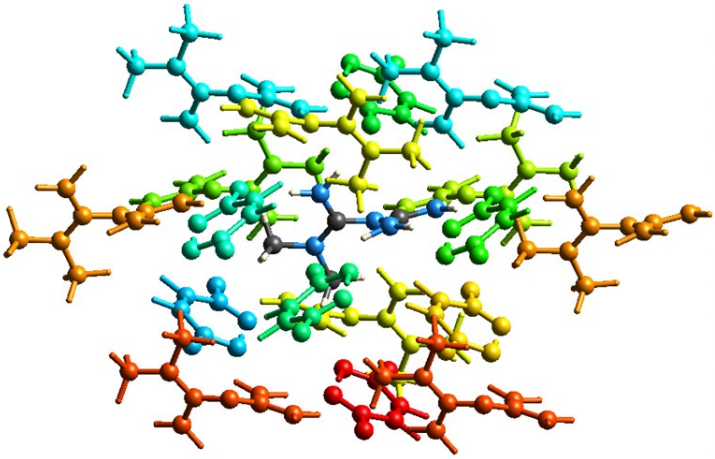


Figure S5. Molecular planes view involved in the cation···anion motif formation of the maleate and malonate metformin salts. The existence of coplanarity between the planes is observed only in the maleate salt structure.



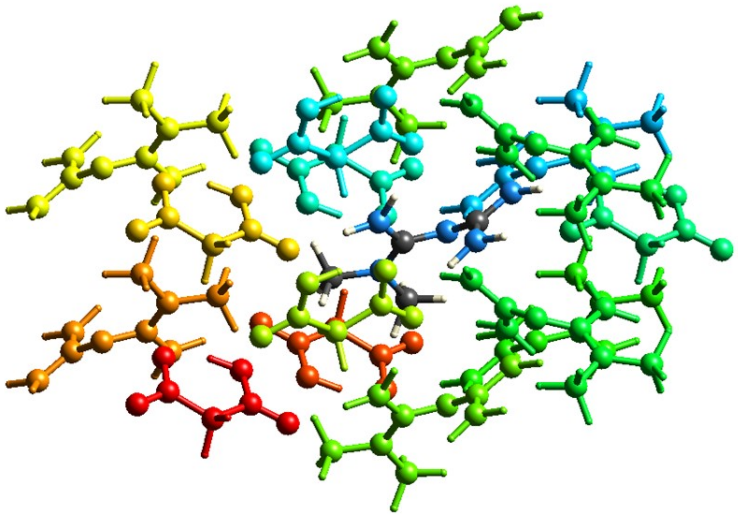
	N	Symop	R	E_ele	E_pol	E_dis	E_rep	E_tot
	2	x, y, z	8.57	-1.6	-0.7	-4.3	2.2	-4.5
	1	-x, -y, -z	9.63	1.5	-0.3	-2.4	0.3	-0.6
	2	x, -y+1/2, z+1/2	7.37	0.0	-1.6	0.0	0.0	-1.2
	1	-x, -y, -z	9.61	2.6	-0.2	-0.9	0.0	1.9
	2	x, y, z	7.92	1.6	-0.2	-2.6	0.2	-0.6
	1	-	5.23	-1.1	-1.6	-12.5	10.5	-6.8
	2	-x, y+1/2, -z+1/2	7.71	-0.1	-0.2	-2.7	0.1	-2.5
	1	-x, -y, -z	8.33	0.6	-0.2	-2.9	0.3	-1.9
	1	-	5.48	1.5	-0.3	-2.4	0.3	-0.6
	2	x, -y+1/2, z+1/2	4.18	-36.6	-18.1	-40.3	72.3	-42.5
	1	-	4.54	-1.3	-0.1	-0.6	0.0	-1.9
	1	-	5.04	1.6	-0.2	-2.6	0.2	-0.6
	1	-	5.00	2.6	-0.2	-0.9	0.0	1.9
	1	-x, -y, -z	5.66	-2.4	-1.0	-24.2	15.3	-14.9
	1	-	5.61	-2.4	-1.0	-24.2	15.3	-14.9
	1	-	5.61	-2.4	-1.0	-24.2	15.3	-14.9
	1	-	5.04	1.6	-0.2	-2.6	0.2	-0.6
	1	-	5.23	-1.1	-1.6	-12.5	10.5	-6.8
	1	-	5.00	2.6	-0.2	-0.9	0.0	1.9
	1	-	5.48	1.5	-0.3	-2.4	0.3	-0.6
	1	-	4.54	-1.3	-0.1	-0.6	0.0	-1.9

Figure S6. Interaction energies for MET-HCl salt calculated with B3LYP model. *R* is the distance (Å) between molecular centroids (mean atomic position) and *N* is the number of times the interaction appears within a radius of 3.8Å from the MET^+ cation (uncolored molecule).



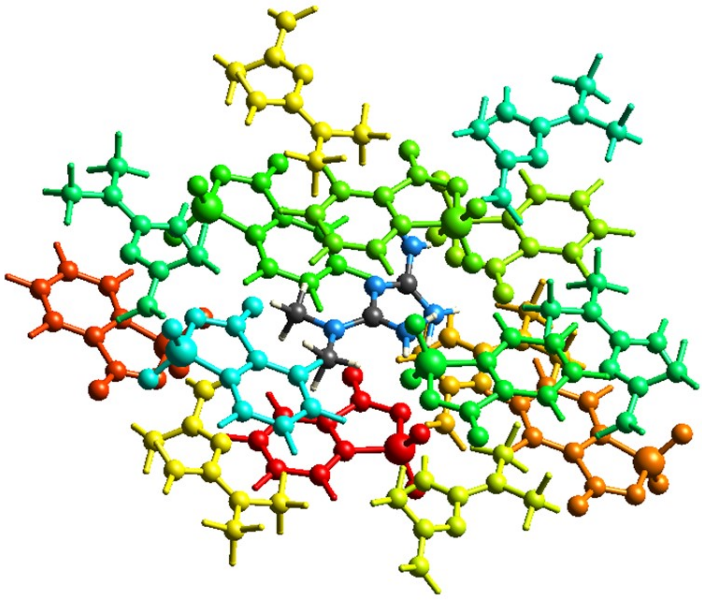
	N	Symop	R	E_ele	E_pol	E_dis	E_rep	E_tot
	1	-	6.03	-1.3	-0.3	-6.9	4.1	-5.1
	2	-x+1/2, y+1/2, -z+1/2	7.93	-0.7	-0.1	-1.6	0.0	-2.2
	2	x, y, z	8.89	0.9	-0.5	-7.0	5.4	-2.2
	1	-	5.28	-11.1	-3.3	-17.1	11.6	-21.9
	2	x+1/2, -y+1/2, z+1/2	7.24	-1.7	-0.3	-6.4	4.4	-4.8
	1	-x, -y, -z	5.71	-64.9	-19.5	-21.1	77.7	-53.4
	1	-x, -y, -z	5.91	-7.8	-3.7	-22.8	8.8	-25.3
	1	-	6.81	-32.3	-3.8	-11.3	65.4	-6.5
	1	-	5.05	-19.3	-3.2	-16.5	27.3	-20.3
	1	-	5.63	-13.1	-2.8	-17.0	44.7	-3.1
	1	-	6.09	-12.9	-2.3	-11.7	29.3	-7.4
	2	-x+1/2, y+1/2, -z+1/2	7.26	-1.8	-0.6	-6.4	1.1	-7.2
	1	-	6.28	1.1	-0.8	-11.6	5.2	-6.4
	1	-x, -y, -z	4.21	0.3	-1.9	-14.9	5.9	-10.5
	1	-	6.09	-12.9	-2.3	-11.7	29.3	-7.4
	1	-	5.05	-19.3	-3.2	-16.5	27.3	-20.3
	1	-	5.28	-11.1	-3.3	-17.1	11.6	-21.9
	1	-	5.63	-13.1	-2.8	-17.0	44.7	-3.1
	1	-x, -y, -z	6.21	2.0	-0.1	-2.3	0.1	0.1
	1	-	6.81	-32.3	-3.8	-11.3	65.4	-6.5
	1	-	6.28	1.1	-0.8	-11.6	5.2	-6.4
	1	-	6.03	-1.3	-0.3	-6.9	4.1	-5.1

Figure S7. Interaction energies for MET-MAL salt calculated with B3LYP model. *R* is the distance (Å) between molecular centroids (mean atomic position) and *N* is the number of times the interaction appears within a radius of 3.8Å from the MET^+ cation (uncolored molecule).



	N	Symop	R	E_ele	E_pol	E_dis	E_rep	E_tot
	1	-	7.95	-0.0	-0.1	-1.7	0.3	-1.4
	1	-	6.21	-0.6	-1.3	-10.5	3.6	-8.5
	1	-x, -y, -z	9.27	-0.9	-0.1	-8.2	5.5	-4.8
	1	-	6.69	-16.8	-2.4	-9.9	33.6	-7.4
	1	-x, -y, -z	9.30	-1.0	-0.1	-1.2	0.0	-2.1
	1	-	5.02	-8.3	-1.8	-15.6	30.2	-5.1
	2	x, y, z	5.01	3.9	-2.9	-17.4	5.9	-9.6
	1	-x, -y, -z	5.40	-0.6	-2.5	-22.3	16.3	-11.9
	2	-x, y+1/2, -z+1/2	7.58	-0.4	-0.7	-8.2	4.2	-5.5
	1	-	6.66	-36.2	-4.2	-11.8	70.1	-8.3
	1	-	5.71	-1.0	-0.6	-8.0	3.7	-6.3
	1	-	5.64	-2.7	-2.8	-14.3	27.0	-0.7
	1	-x, -y, -z	5.46	-61.7	-19.6	-27.6	87.0	-50.0
	2	x, y, z	5.01	0.9	-1.4	-7.0	3.9	-3.8
	2	-x, y+1/2, -z+1/2	4.81	-13.9	-2.9	-14.9	13.9	-21.2
	1	-	5.64	-2.7	-2.8	-14.3	27.0	-0.7
	1	-	6.21	-0.6	-1.3	-10.5	3.6	-8.5
	1	-	6.66	-36.2	-4.2	-11.8	70.1	-8.3
	1	-	5.02	-8.3	-1.8	-15.6	30.2	-5.1
	1	-	5.71	-1.0	-0.6	-8.0	3.7	-6.3
	1	-	6.69	-16.8	-2.4	-9.9	33.6	-7.4
	1	-	7.95	-0.0	-0.1	-1.7	0.3	-1.4

Figure S8. Interaction energies for MET-MLN salt calculated with B3LYP model. *R* is the distance (Å) between molecular centroids (mean atomic position) and *N* is the number of times the interaction appears within a radius of 3.8Å from the MET^+ cation (uncolored molecule).



	N	Symop	R	E_ele	E_pol	E_dis	E_rep	E_tot
	1	-	4.14	-14.8	-2.9	-41.3	37.6	-30.5
	1	-	7.98	0.4	-0.9	-5.2	4.9	-1.8
	1	-	8.65	4.3	-0.9	-5.2	1.7	0.4
	1	-x, -y, -z	7.21	-5.6	-1.0	-8.0	4.9	-10.7
	2	x+1/2, -y+1/2, z+1/2	8.26	-1.0	-0.3	-5.9	1.8	-5.4
	1	-x, -y, -z	8.18	1.3	-0.2	-2.3	0.0	-0.7
	1	-	7.25	-30.5	-5.0	-14.5	59.1	-12.1
	1	-	3.84	-21.1	-6.6	-41.1	33.1	-42.5
	1	-	6.69	-6.8	-2.9	-15.2	6.6	-18.5
	1	-	6.62	-14.3	-3.3	-10.4	25.3	-11.0
	2	-x+1/2, y+1/2, -z+1/2	7.83	-1.0	-0.2	-4.5	1.3	-4.3
	1	-x, -y, -z	8.32	-4.6	-1.2	-7.8	6.8	-8.3
	1	-	7.99	-1.6	-1.7	-10.5	13.9	-3.6
	1	-x, -y, -z	7.42	-1.4	-0.6	-2.2	0.1	-3.7
	2	x+1/2, -y+1/2, z+1/2	8.62	-7.9	-1.9	-5.7	7.8	-9.9
	1	-	4.14	-14.8	-2.9	-41.3	37.6	-30.5
	1	-	6.69	-6.8	-2.9	-15.2	6.6	-18.5
	2	-x+1/2, y+1/2, -z+1/2	7.93	-8.1	-1.3	-7.8	4.0	-13.8
	1	-	8.65	4.3	-0.9	-5.2	1.7	0.4
	1	-x, -y, -z	6.49	-20.8	-3.1	-11.1	12.8	-26.1
	1	-	6.62	-14.3	-3.3	-10.4	25.3	-11.0
	1	-	7.25	-30.5	-5.0	-14.5	59.1	-12.1
	1	-	3.84	-21.1	-6.6	-41.1	33.1	-42.5
	1	-	7.99	-1.6	-1.7	-10.5	13.9	-3.6
	1	-	7.98	0.4	-0.9	-5.2	4.9	-1.8

Figure S9. Interaction energies for MET-SAC salt calculated with B3LYP model. *R* is the distance (Å) between molecular centroids (mean atomic position) and *N* is the number of times the interaction appears within a radius of 3.8Å from the MET^+ cation (uncolored molecule).

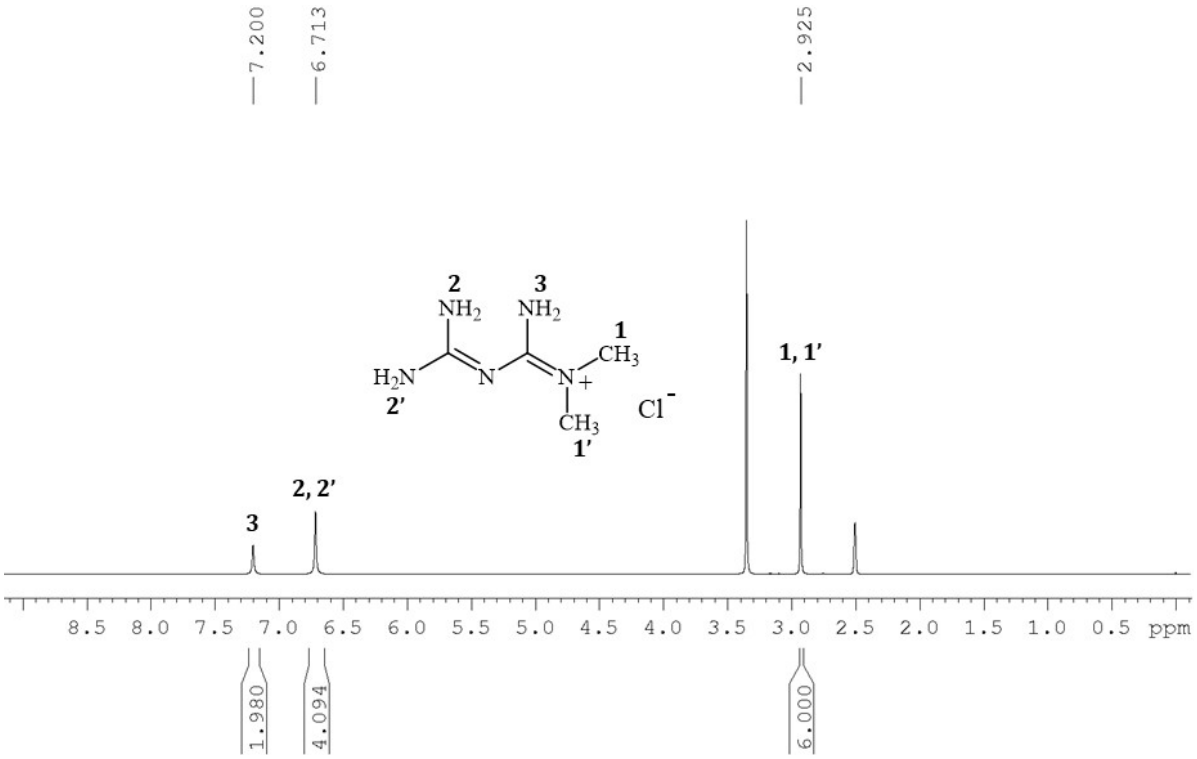


Figure S10. [1]H NMR (400 MHz) spectrum of metformin hydrochloride (MET-HCl) in DMSO-d_6.

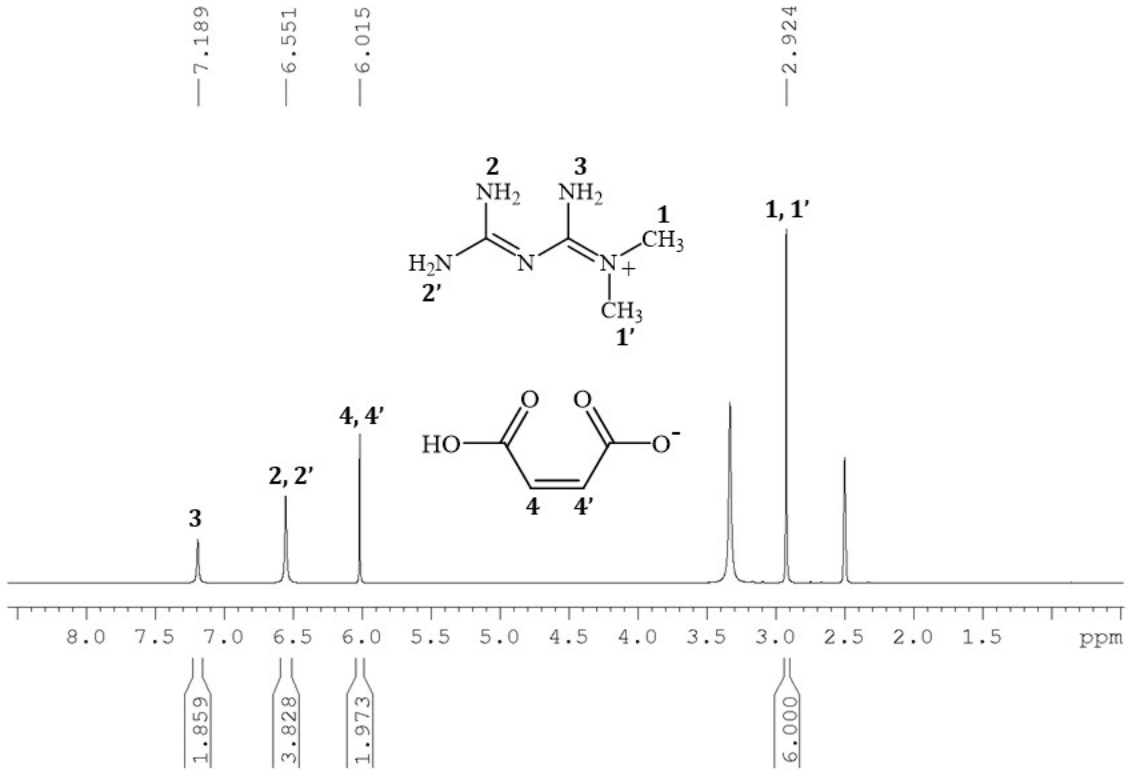


Figure S11. [1]H NMR (400 MHz) spectrum of metformin maleate (MET-MAL) in DMSO-d_6.

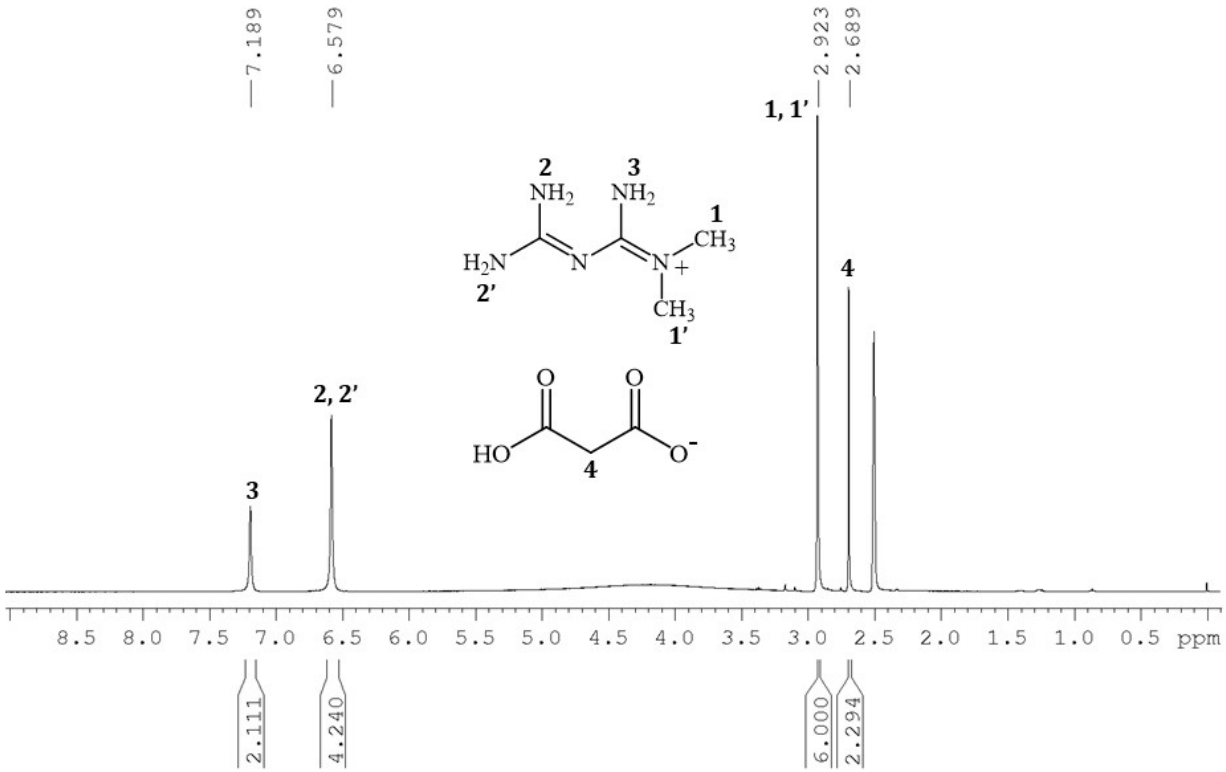


Figure S12. ^{1}H NMR (400 MHz) spectrum of metformin malonate (MET-MLN) in DMSO-d_6.

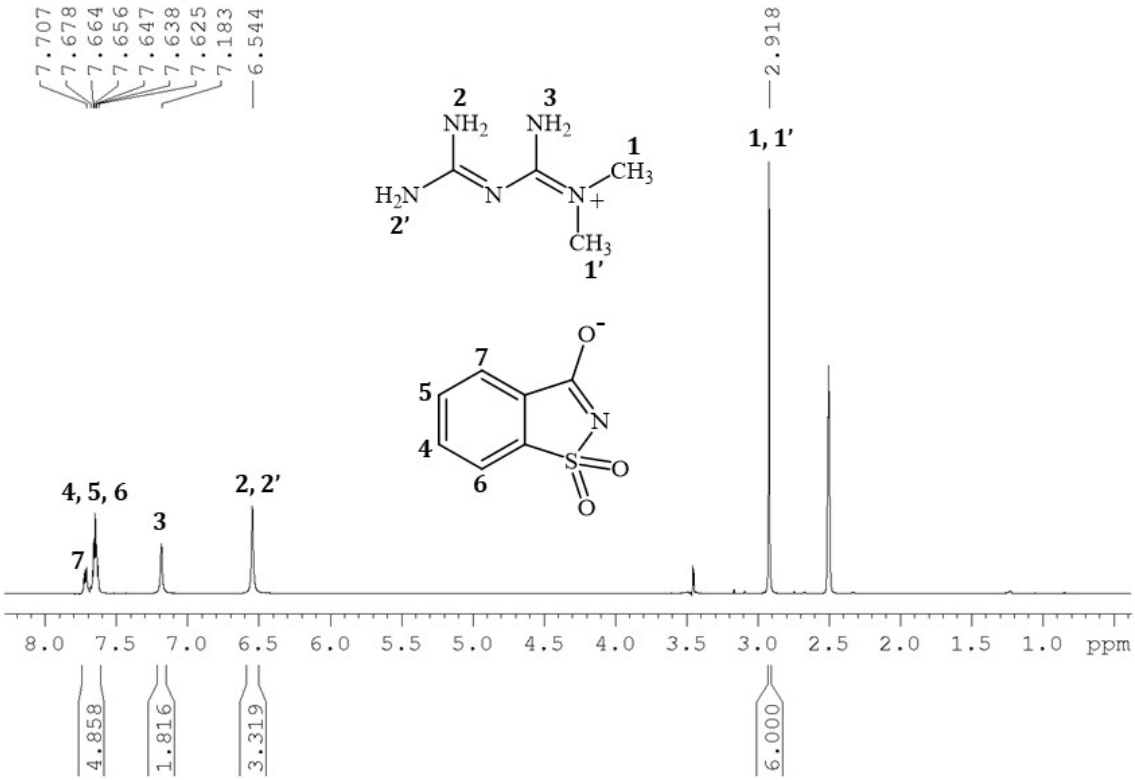


Figure S13. ^{1}H NMR (400 MHz) spectrum of metformin saccharinate (MET-SAC) in DMSO-d_6.

Table S1. Results of linearity, accuracy and precision obtained during validation of the analytical method.

Parameter	Results
Linearity	
Concentration range (μg mL^{-1})	8.0-40.0
Regression	$F = 74389$ ($p < 0.001$)
Lack of fit	$F = 2.64$ ($p = 0.10$)
Slope (b)	111531
Intercept (a)	-47289
Coefficient of determination (R^2)	0.9998
Accuracy	
Level 1 (8.0 μg mL^{-1})	Mean recovery: 99.98% (n = 6)
Level 2 (24.0 μg mL^{-1})	Mean recovery: 100.03 (n = 6)
Level 3 (40.0 μg mL^{-1})	Mean recovery: 100.25 (n = 6)
Precision	
Repeatability (RSD%)	0.27-0.83 (n = 3)
Intermediate (RSD%)	0.37-0.64 (n = 6)

RSD, relative standard deviation.

Table S2. Parameters of the calibration curves obtained during validation of the bioanalytical method.

Day of analysis	Linearity range (ng mL^{-1})	Linear regression		
		Slope (b)	Intercept (a)	R^2
First	200-1400	108.19	-1031	0.9995
Second		108.24	-1079	0.9997
Third		108.78	-1173	0.9998

R^2, coefficient of determination.

Table S3. Precision, accuracy, and recovery assays performed during validation of the bioanalytical method.

Level	Nominal concentration (ng mL^{-1})	Within-run (n = 5)			Between runs (n = 15)		
		MMC (ng mL^{-1})	Precision RSD (%)	Accuracy RSE (%)	MMC (ng mL^{-1})	Precision RSD (%)	Accuracy RSE (%)
LLOQ QC	200	194.6	2.06	-2.71	200.2	3.71	0.09
LQC	500	490.2	1.90	-1.95	488.6	1.26	-2.28
MQC	800	801.4	0.24	0.18	784.6	1.75	-1.92
HQC	1100	1107.0	0.32	0.64	1086.4	1.82	-1.24
DQC	2000	1954.6	0.27	-2.27	1949.6	0.95	-2.52

MMC, mean measured concentration; RSE, relative standard error; RSD, relative standard deviation; LLOQ QC, lower limit of quantification quality control; LQC, low quality control; MQC, mid quality control; HQC high quality control; DQC, dilution quality control.

Table S4. Results of **s**tability assays obtained during validation of the bioanalytical method.

Level	Nominal concentration (ng mL^{-1})	Bench-top stability (room temperature for 1.5 h)		Long-term stability (-70 °C for 4.5 h)		Freeze-thaw stability (three cycles)	
		RSD (%)	RSE (%)	RSD (%)	RSE (%)	RSD (%)	RSE (%)
LQC	500	1.03	-2.75	0.57	-3.61	3.52	-1.72
HQC	1100	0.15	0.46	0.41	1.06	2.77	3.04

RSE, relative standard error; RSD, relative standard deviation; LQC, low quality control; HQC, high quality control.

Table S5. Composition of the solutions and standard buffer solutions used in the solubility and dissolution experiments.

Hydrochloric Acid (HCl) Solution (1000 mL)	
pH	1.2
Conc. (mol/L)	0.1
$HCl_{conc.}$ (mL)	8.4
Deionized water to complete the 1000 mL volumetric flask	
Acetic Acid Solution (1000 mL)	
Conc. (mol/L)	0.2
$CH_3COOH_{glacial}$ (mL)	11.5
Deionized water to complete the 1000 mL volumetric flask	
Sodium Acetate Solution (1000 mL)	
Conc. (mol/L)	0.2
$C_2H_3O_2Na$ (g)	16.4
Deionized water to complete the 1000 mL volumetric flask	
Acetate Buffer (1000 mL)	
pH	4.5
0.2 M $C_2H_3O_2Na$ (mL)	220
0.2 M CH_3COOH (mL)	250
Deionized water to complete the 1000 mL volumetric flask	
Monopotassium Phosphate Solution (1000 mL)	
Conc. (mol/L)	0.2
KH_2PO_4 (g)	27.2
Deionized water to complete the 1000 mL volumetric flask	
Dipotassium Phosphate Solution (1000 mL)	
Conc. (mol/L)	0.2
K_2HPO_4 (g)	34.8
Deionized water to complete the 1000 mL volumetric flask	
Phosphate Buffer (1000 mL)	
pH	6.8
0.2 M KH_2PO_4 (mL)	255
0.2 M K_2HPO_4 (mL)	245
Deionized water to complete the 1000 mL volumetric flask	

Table S6. Calculated ΔpKa between MET and the salt formers.

Compound	pKa	ΔpKa = (pK_a(base) − pK_a(acid))	Stoichiometry
Metformin free base	12.4	-	
Maleic acid	1.93	10.47	1:1 salt
Malonic acid	2.84	9.56	1:1 salt
Saccharin	1.60	10.80	1:1 salt

Table S7. Principal FT-IR bands (cm^{-1}) for the novel MET salts.

MET-MAL	MET-MLN	MET-SAC	Assignment
3350 and 3194	3328, 3149	3344 and 3150	$\nu\,(NH_2)_{amine}$
2936	2937	2930	$\nu\,(CH)_{aliphatic}$
1706	1730	-	$\nu\,(CO)_{carboxyl}$
-	-	1638	$\nu\,(CO)_{amide}$
1604	1589	-	$\nu_a\,(COO^-)_{carboxylate}$
1414	1425	-	$\nu_s\,(COO^-)_{carboxylate}$
-	-	1332 and 1140	$\nu\,(SO_2)_{sulphonyl}$

v = stretching; *a* = antisymmetric; *s* = symmetric.

Table S8. pH measured in different dissolution media before and after solubility studies.

Dissolution media	pH 6.8	pH 4.5	pH 1.2	H_2O
pH (dissolution media before the tests)	6.80	4.50	1.20	5.90
pH (MET-HCl solution)	6.68	4.41	1.23	3.35
pH (MET-MAL solution)	6.73	4.45	1.26	3.58
pH (MET-MLN solution)	6.75	4.47	1.25	3.64
pH (MET-SAC solution)	6.71	4.43	1.27	3.53