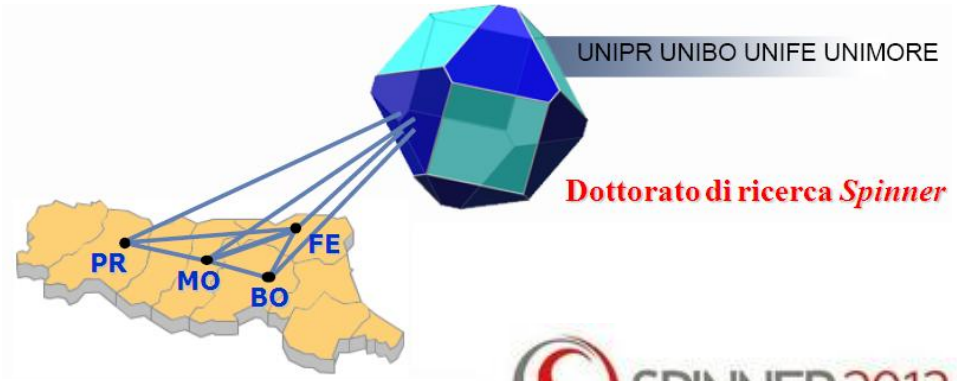
The role of co-crystallization screening for the assessment of structure-activity relationship in drug development



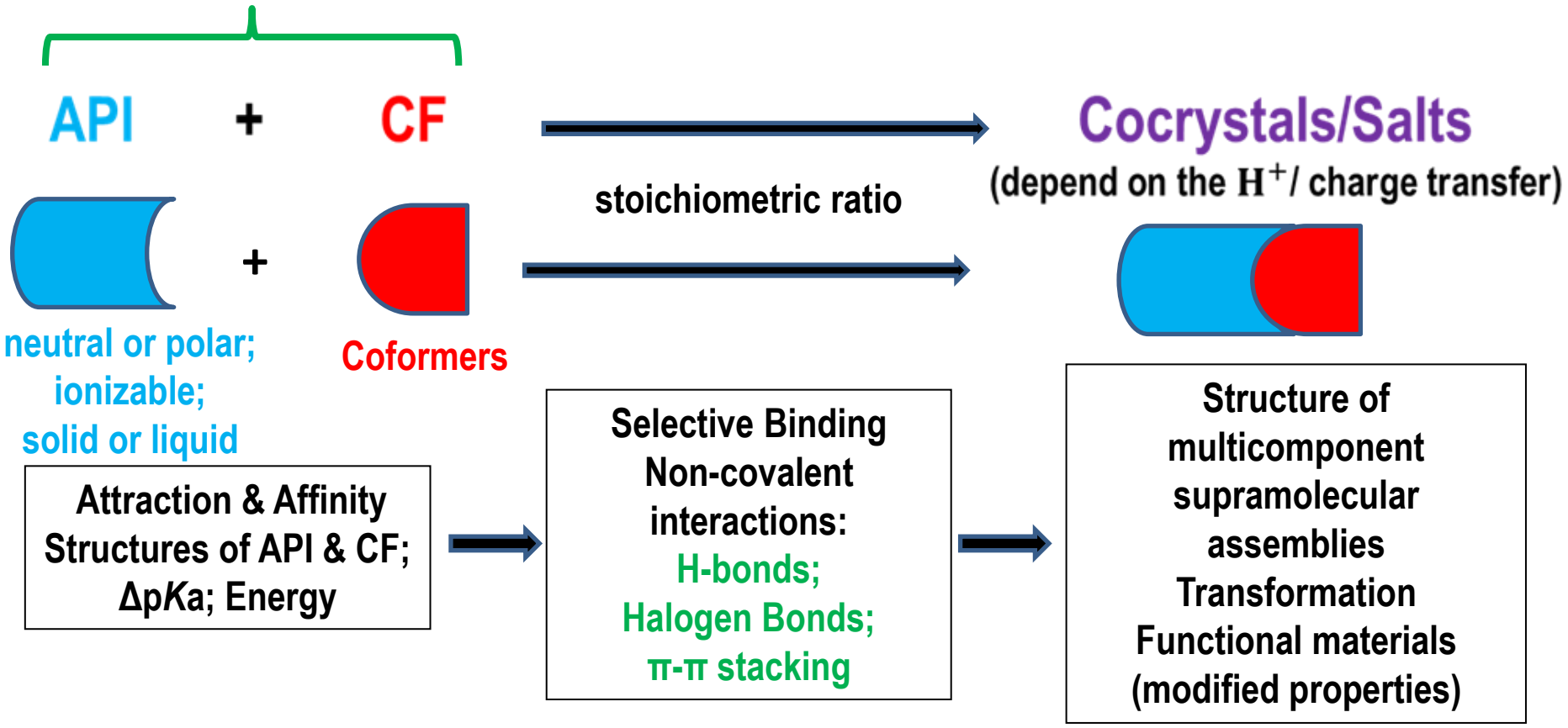
УНИВЕРЗИТЕТ
„ГОЦЕ ДЕЛЧЕВ“
ШТИП

Aleksandar Cvetkovski, PhD

Faculty of Medical Sciences, University Goce Delcev, Stip

Ohrid, 03/06/2016

What are cocrystals?



Driving forces for molecular cocrystal formation

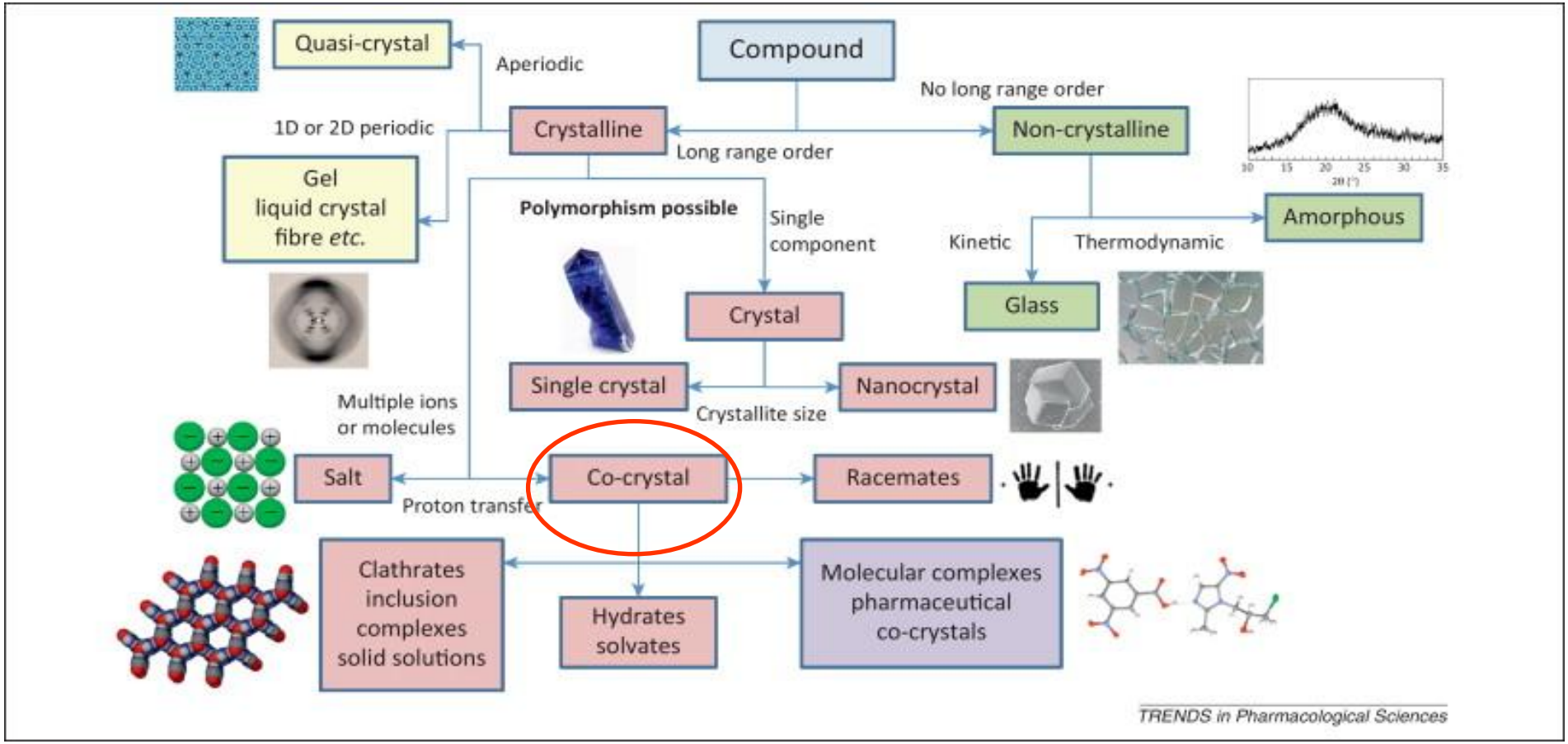
"Molecular recognition is selective binding with a purpose"

J.-M. Lehn, *Pure Appl. Chem.* 1978, *50*, 871.

FDA Guidance for Industry Regulatory Classification of Pharmaceutical Co-Crystals, 2013

Where is the place of Molecular Cocrystals among the Solid Forms?

Cocrystals are solid crystalline single phase materials composed of two or more different molecular and/or ionic compounds, generally in a stoichiometric ratio.



Aitipamula, S. *et al.* , Polymorphs, Salts, and Cocrystals: What's in a Name?, *Cryst. Growth Des.* 2012, *12*, 2147−2152

Pharmaceutical Co-crystals

1: 1 Caffeine – Citric acid (apnea treatments in newborn) **J.Chem.Crystallogr (2015) 45:128-133)**

Aminophylline
2:1:1 Theophylline – Ethylendiamine – Water (***Mol. Pharmac.*** **VOL. 4, NO. 3, 323-338**)

pTeroPure®, potent antioxidant, energizer
1:1 Caffeine – Pterostilbene , ***CrystEngComm*****, 2010,12, 2436-2442**

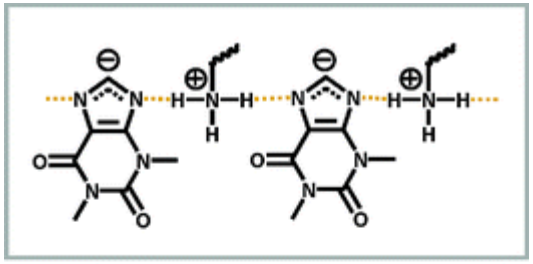
Itraconazole – ITZ (Sporanox, Janssen-Cilag, antimictic)
1:1 ITZ Amorphous form – 1,4dicarboxylic acids (succinic acid, L-tartaric acid or L-malic acid) ***Adv. Drug Deliv. Rev.*** **56, 275-300 (2004)**

Carbamazepine- CBZ (Tegretol, Novartis, anticonvulsant)
1:1 CBZ polymorphyc form III – Saccharin ***Eur. J. Pharm. Biopharm*****. 67, 112-119 (2007)**

R,S Pregabalin – PREG (Lyrica, Pfizer; neuropathic pain)
1:1 (S)-pregabalin – (S)-Mandelic acid ***Act.Crystall.*** **Section E 63(10),2007**

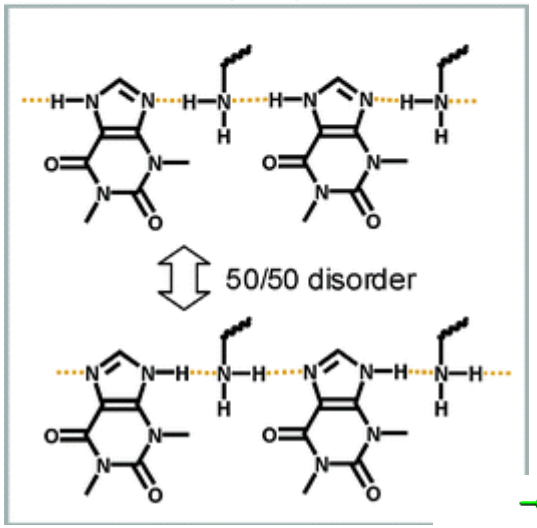
Paracetamol- PAR (Panadol, GlaxoSmithKline, pain killer)
1:1 PAR polymorph I – Oxalic acid ***Adv.Materials*** **21(38-39):3905 - 3909 · July 2009**

Aminophylline (charge/ H^+ transfer)

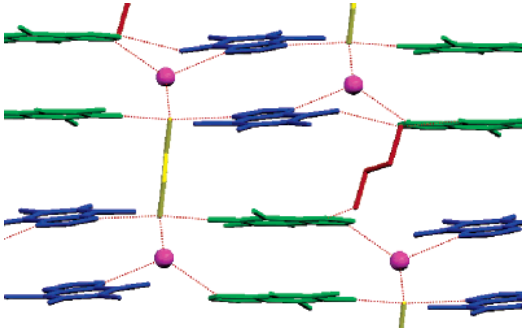


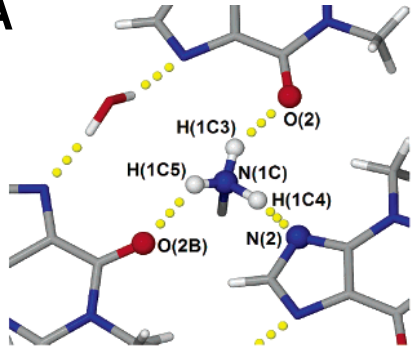
75% total occupancy – ionized

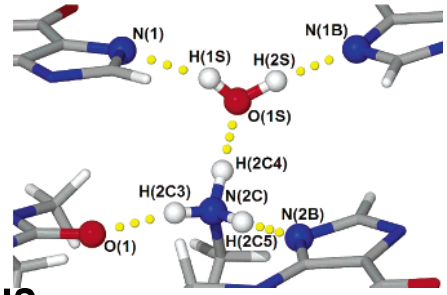
25% total occupancy – non-ionized



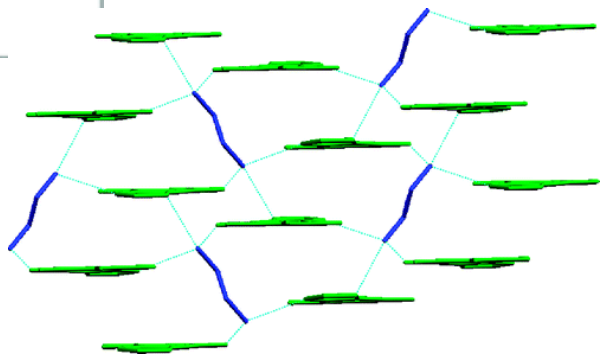
Aminophylline hydrate
2 : 1 THEO : 1 EDA

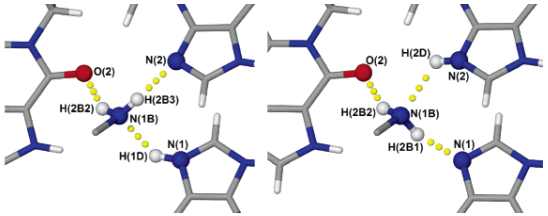






Aminophylline anhydrous
2 : 1 THEO : EDA

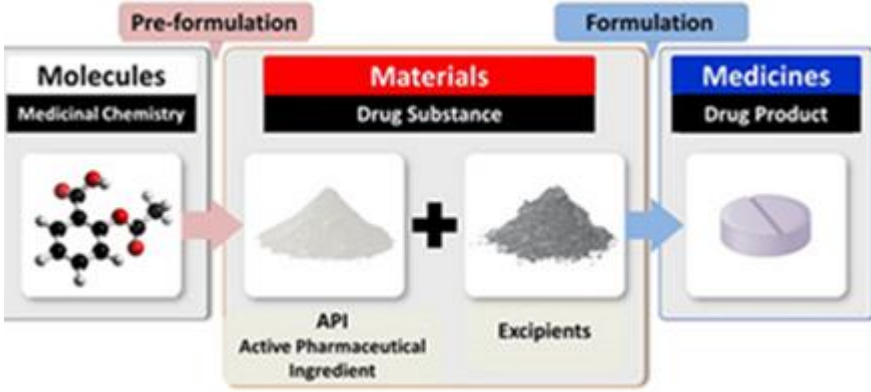




Mol. Pharmacy VOL. 4, NO. 3, 323-338

What are the advantages of drug co-crystals?

- Improving the solubility/ controlling the dissolution patterns (drugs of BCS Class III);
- Controlling the morphology (crystal size & shape) and melting point;
- Controlling the phase transition in the solid state (polymorphs, hydrates/ solvates)
- Improving the API's biopharmaceutical profile (e.g. avoiding the common salt effect in GI)



- Enhance the chemical stability
- IPR/ Patent protection/ Extend the drug life-time (either as generic or NCE)
- Development of "drug-drug" type of CC for "fixed-dose" combo formulations
- Stereo selective resolution of racemic APIs
- Green Chemistry Principles/ Eco-Friendly methods of preparation

Objectives for the Research on Cocrystals

- **Cocrystallization screening:**

  Drug model: - Metformin (MET): *N,N*- dimethylbiguanide (1st choice in oral antidiabetic therapy) ;
  - first line drug in oral therapy of diabetes type-2 anticancer activity
  - anticancer activity *(Cancer Treat Res. 2014;159:355-76)*

  CC formers/ Ligands: - compounds from U.S. FDA's GRAS list
  http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/SCOGS/default.htm
  - approved status of salt counter-ions by the FDA; annex in:
  Serajuddin & Pudipeddi, Handbook of Pharmaceutical Salts, IUPAC, 2002

- **To develop protocol for isolation and crystallization of the neutral form of MET and molecular salts of mono- and di- protonated MET;**

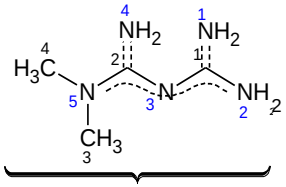
- **To determine structure of the crystalline samples of PCCs with quality for single-crystal X-ray diffraction analyses & Analysis of the molecular geometry and of the crystal packing for identifying intermolecular interactions;**

- **To optimize the Cocrystallization method for selected samples of MET PCCs with resolved structures (scalability and reproducibility of the batches of PCCs)**

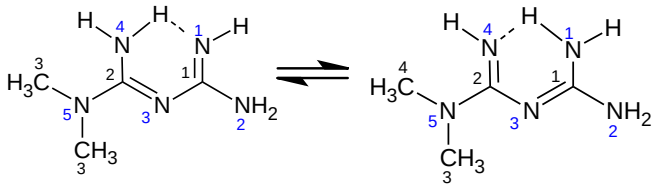
- **To study structure – activity relationship in "drug-drug" type of PCC (ex. MET – DCC 1:1 & 1:2 ratio in PCC**

Co- crystallozation of MET with 25 CFs

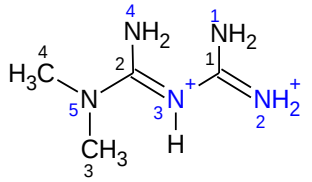
Monoprotonated MET



Neutral MET



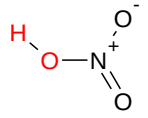
Diprotonated MET

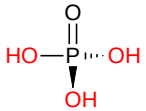


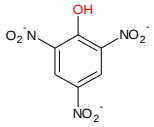
Metformin = L; $[HL]/[L][H]$ $pK_{a1}(N{-}H^+)$ ~ 12.40; $[H_2L]/[HL][H]$ $pK_{a2}(N{-}H^+)$ = 2.96 (NIST database) *Source*:

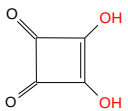
Hariharan, et al., 1989, *ActaCryst.* C45
Childs, et al., 2004, *Cryst.Growth.Des.* 4, 3

Strong acids

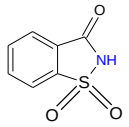

Nitric Acid

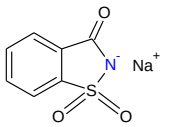

Phosphoric acid

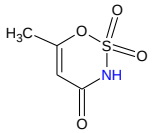

Picric Acid

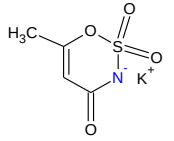

Squaric acid

Functional excipients (artificial sweeteners)

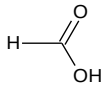

Saccarine

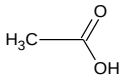

Na Saccharinate

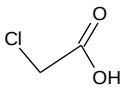

Acesulfame

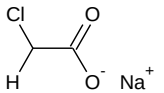

Acesulfame K

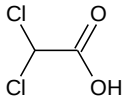
Monocarboxylic acids

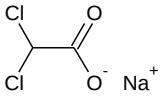

Formic acid

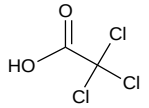

Acetic acid

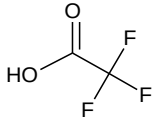

Chloroacetic acid

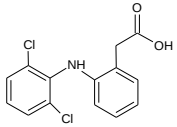

Na chloroacetate

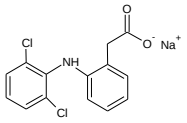

Dichloroacetic acid

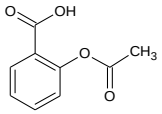

Na dichloroacetate

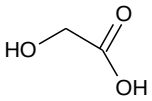

Trichloroacetic acid


Trifluoroacetic acid


Diclofenac

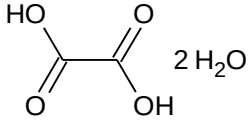

Na Diclofenac

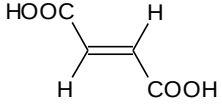

Acetylsalicylic acid

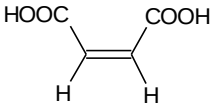

Glycolic Acid

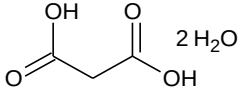
API (Active Pharmaceutical Ingredients); *cosmeceuticals*

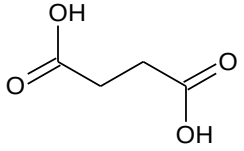
Dicarboxylic acids

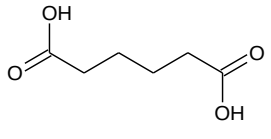

Oxalic acid dihydrate


Fumaric acid


Maleic acid

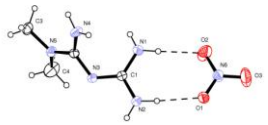

Malonic acid hydrate


Succinic acid

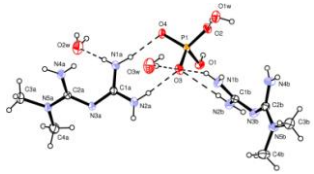

Adipic acid

Structures determined: 25 molecular co-crystals of MET

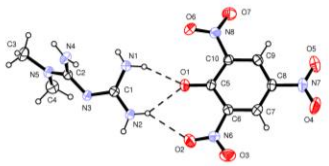
MET – Nitric acid 1:1



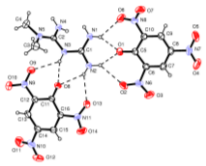
ME – phosphoric acid trihydtrate 2 :1:3



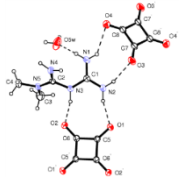
MET – Picric acid1:1



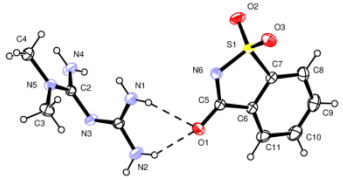
MET – Picric acid 1:2



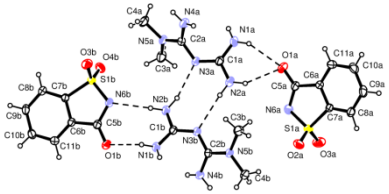
MET – SQA hydrate 1:1:1



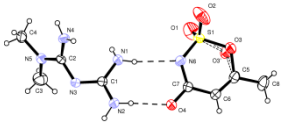
MET – Saccharine 1:1, Polymorph I



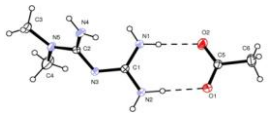
MET – Saccharine 1:1, Polymorph II



MET – Acesulfame 1:1

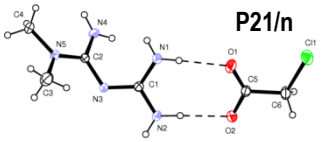


MET – Acetic acid 1:1



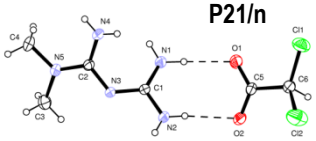
MET – Chloroaceti c acid 1:1

P21/n



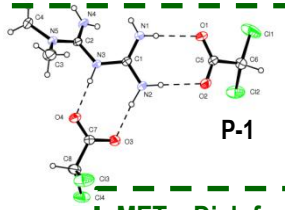
MET – Dichloroacetic acid 1:1

P21/n



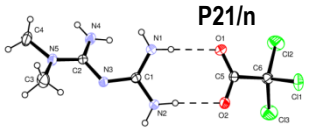
MET – Dichloroacetic acid 1:2

P-1



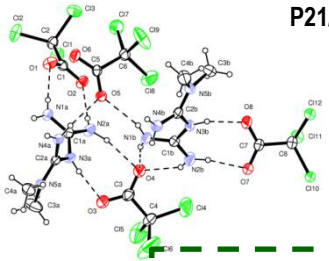
MET – Trichloroacetic acid 1:1

P21/n



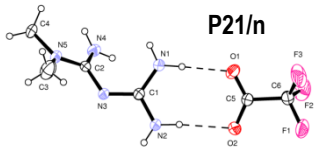
MET – Trichloroacetic acid 1:2

P21/a

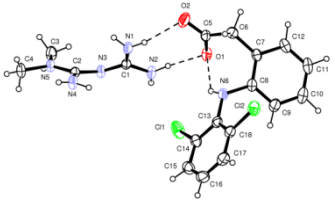


MET – Trifluoroacetic acid 1:1

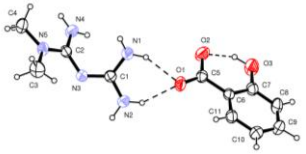
P21/n



MET – Diclofenac1:1

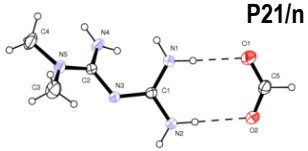


MET – Salicylic acid 1:1

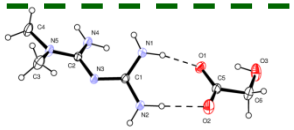


MET – Formic acid 1:1

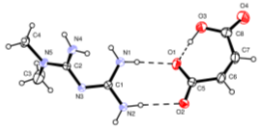
P21/n



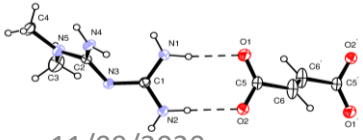
MET – Glycolic acid 1:1



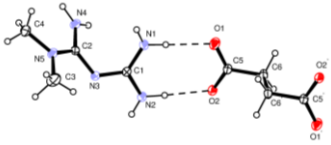
MET - Maleic acid 1:1



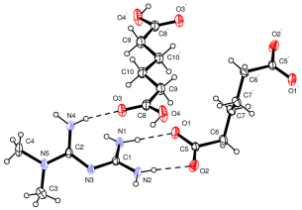
MET – Fumaric acid 1 : 0.5



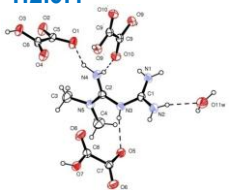
MET –Succinic acid 1 : 0.5



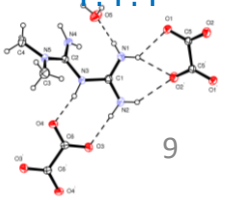
MET – Adipic acid 1 : 1



MET – Oxalic acid hydrate 1:2.5:1



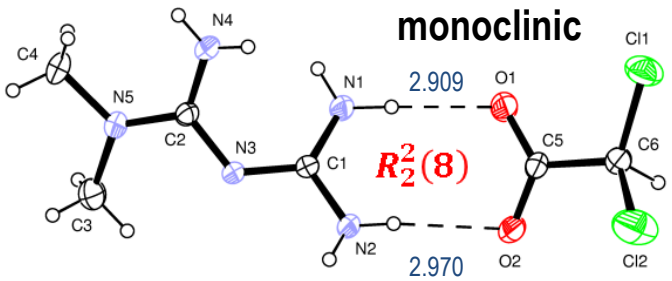
MET – Oxalic acid hydrate 1 : 1 : 1



Packing motifs in "*drug-drug*" CCs MET – DCA

MET – DCA 1:1

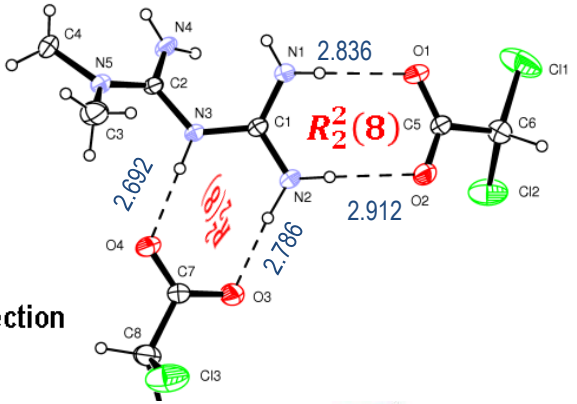
P21/n
monoclinic

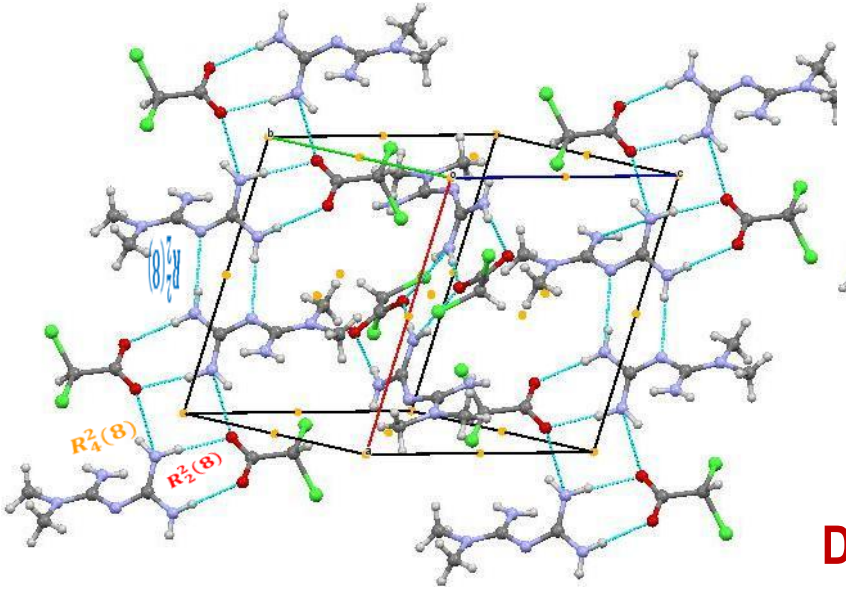


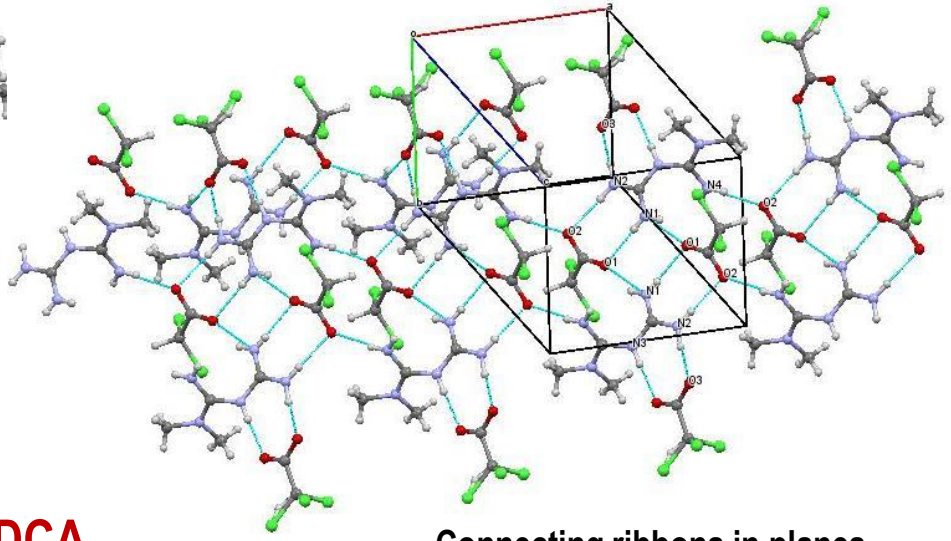
$R_2^2(8)$ N–H···O dimer
$R_2^2(8)$ N–H···N dimer
$R_4^2(8)$ N–H···O tetramer
N4–H1···O2; ribbon connection

MET – DCA 1: 2

P -1
triclinic







DCA

Anti-hyperglycemic; anti-cancer drug

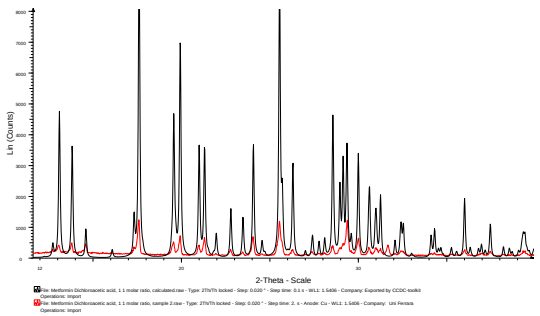
(Papandreu *et.al.*, *Int.J.Cancer*:128, 1001-1008 (2011)

Connecting ribbons in planes
N4–H1···O2
N4–H3···O3

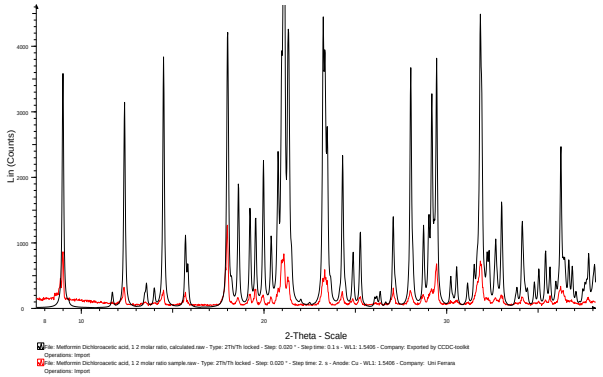
Quality Control of the Scalable Method of Preparation of the MET – DCA 1:1 and MET – DCA 1:2

XRPD patterns

a

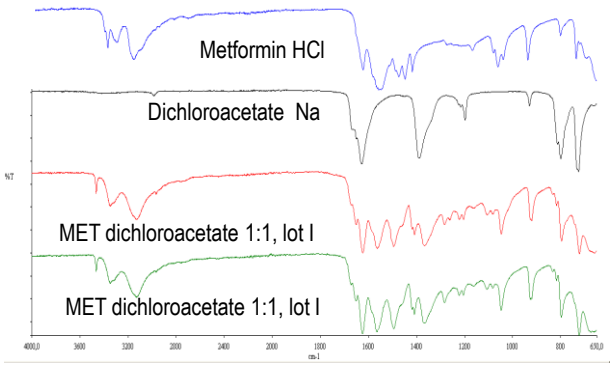


b

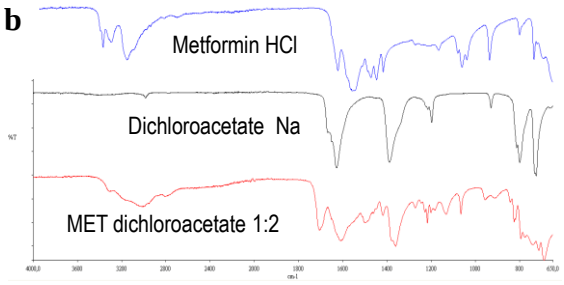


FT-IR spectra

a

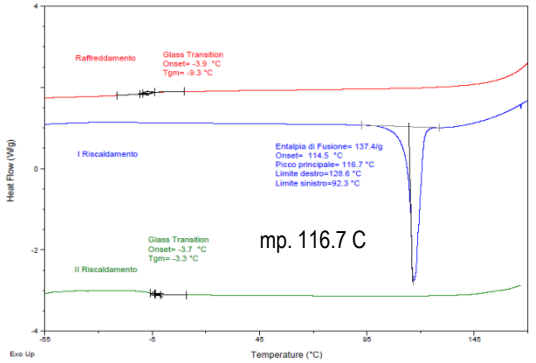


b

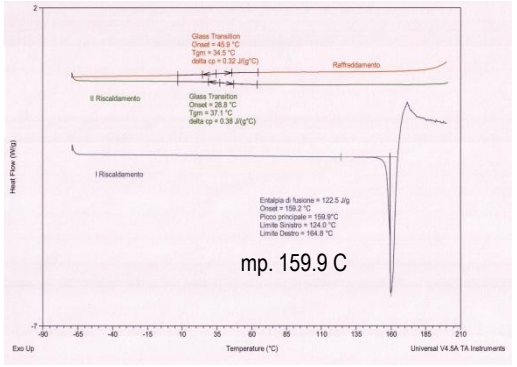


DSC Thermograms

a



b



MET-DCA 1:1 (a)

MET-DCA 1:2 (b)

Preliminary data of the preclinical testing of MET – DCA molecular cocrystals

In vitro **cytotoxic activity of MET – DCA 1:1 and MET-DCA 1:2 measurements on EHEB cell line (wild type B)**

Esp. 30-3-15 EHEB	*EHEB*	
	Vitality	
Treatments	24h	48h
UNT	100	100
Metformin 15000uM	81	52
Metformin 1500uM	88	78
Metformin 150uM	95	86
DCA 15000uM	53	54
DCA 1500uM	86	89
DCA 150uM	100	97
Met-DCA 1:1 30000uM sale	31	5
Met-DCA 1:1 3000uM sale	69	51
Met-DCA 1:1 300uM sale	82	80
Met 15000uM + DCA 15000uM mix	40	16
Met 1500uM + DCA 1500uM mix	83	59
Met 150uM + DCA 150uM mix	94	87
UNT	100	100
Metformin 10000uM	97	58
Metformin 1000uM	99	86
Metformin 100uM	100	91
DCA 20000uM	51	41
DCA 2000uM	77	87
DCA 200uM	94	91
Met-DCA 1:2 30000uM sale	18	4
Met-DCA 1:2 3000uM sale	61	43
Met-DCA 1:2 300uM sale	67	76
Met 10000uM + DCA 20000uM mix	30	15
Met 1000uM + DCA 2000uM mix	72	66
Met 100uM + DCA 200uM mix	74	91

(***submitted for publishing***)

Conclusions & Further Perspectives

- ✓ **Crystallography, method of first choice for structure determination, is the best for crystallization screening, and thus reveling the intermolecular interactions between API-API and API-excipients**
- ✓ **Complex packing motifs in MET CCs imply the need of systematic study on molecular recognition phenomena at the early stage of the drug development process (isolation & characterization of the unique single, but multicomponent crystalline phases);**
- ✓ **MET – DCA in 1:1 and 1:2 ratio offer opportunities for studying anti hyperglycemic and anticancer synergistic activity**
- ✓ **"Drug-Drug" type of CC (MET – DCA) may be used for designing "combo"/ fixed dosed pharmaceutical formulations**

Thank you for attention

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