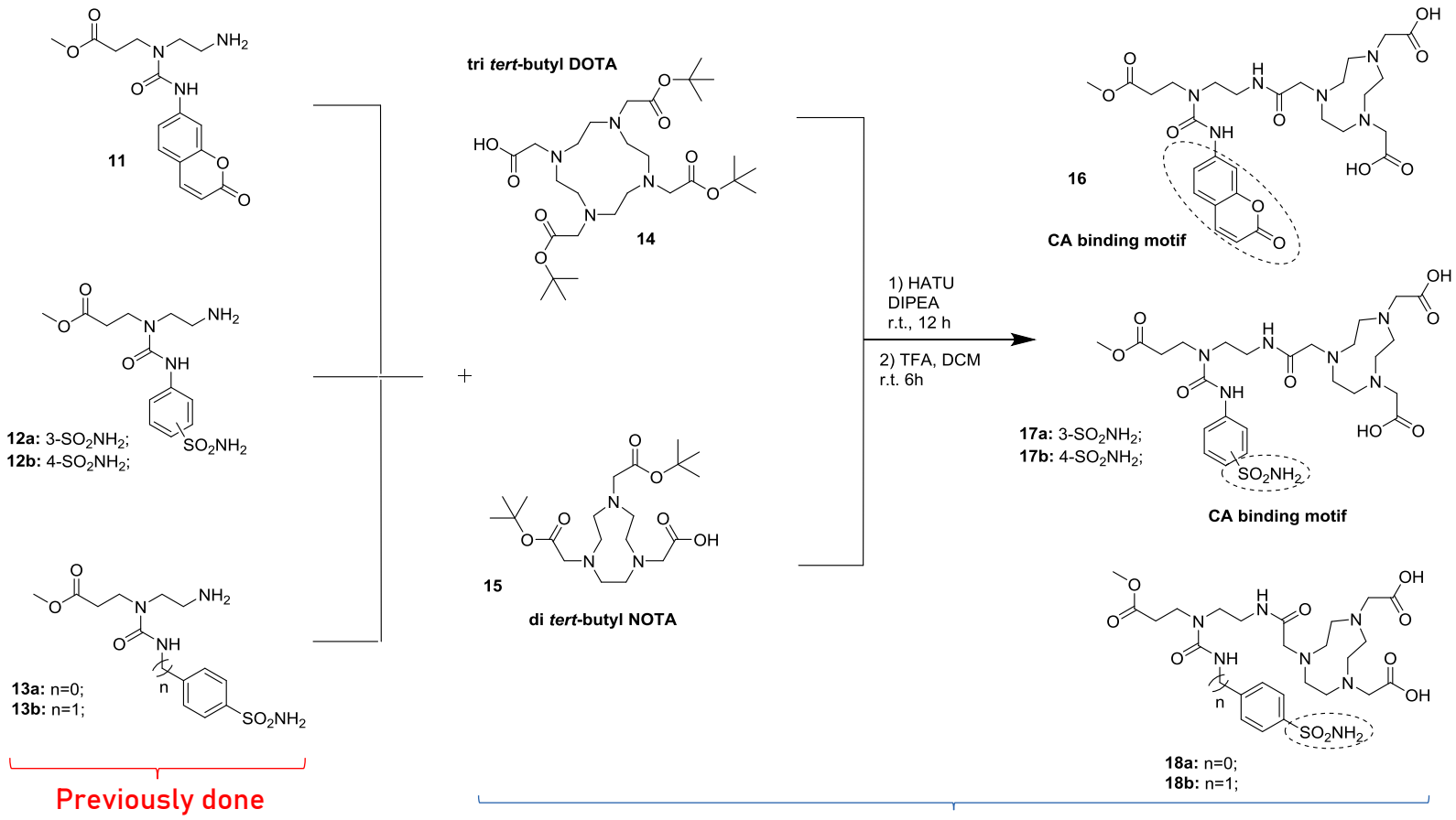
Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA
UNIVERSITÀ DEGLI STUDI FIRENZE

Design and synthesis of 3 series of mono and multimeric radiotracers targeting tumor microenvironment and designed as selective theranostic agents

University of Florence, Department of NEUROFARBA and DICUS

Pisa 12/12/2024

Synthesis of mono and dual inhibitors against tumor-associated human Carbonic Anhydrases and FAP-α: where were we 1 year ago?



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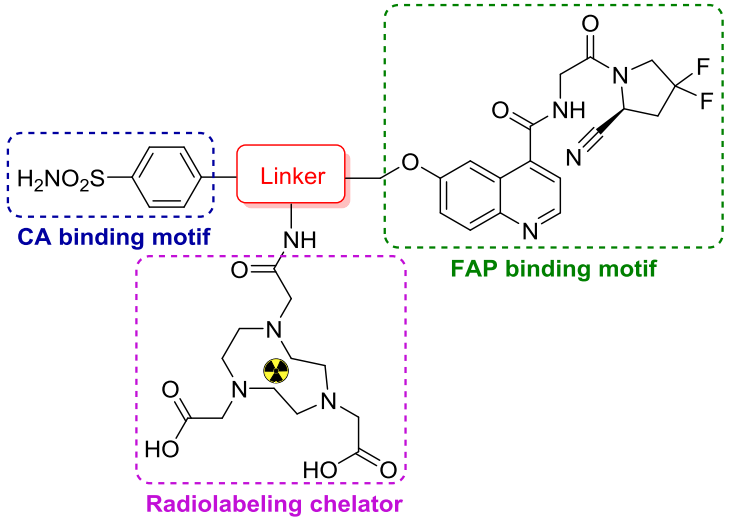
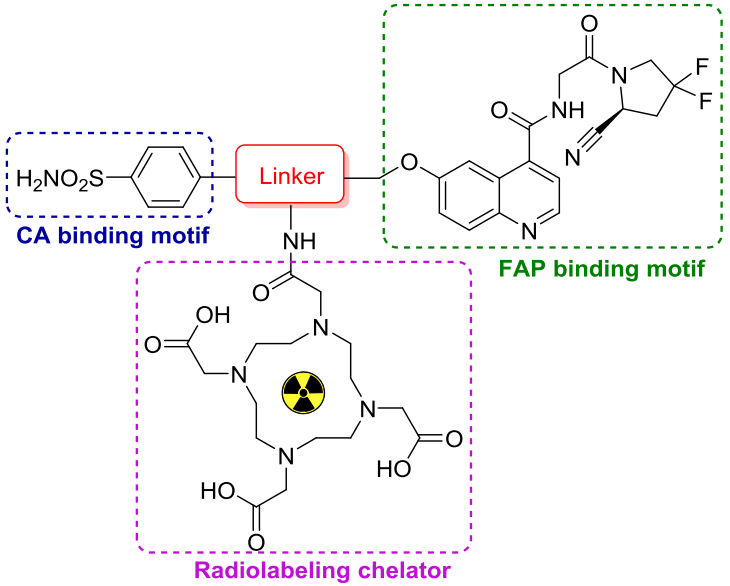
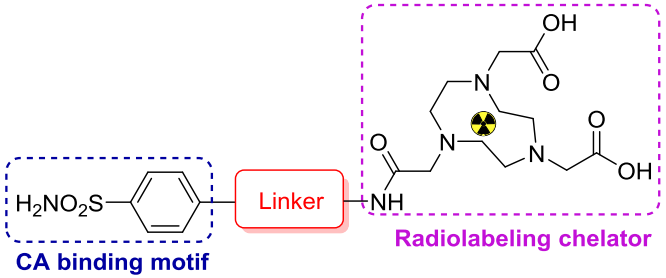
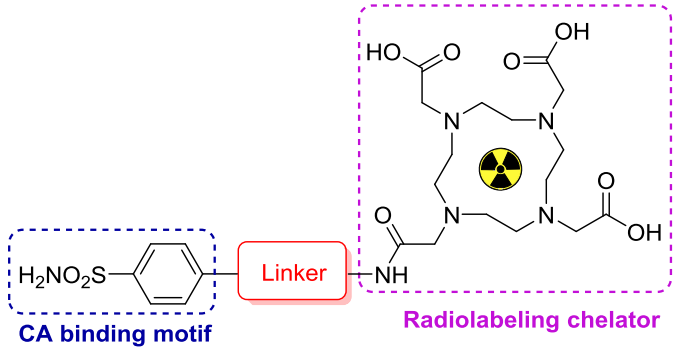
Design and Synthesis of mono and dual inhibitors against tumor-associated human Carbonic Anhydrases and FAP-α: where are we now?

- 2 Different series of dual inhibitors coupled with radiolabeling chelator were synthesized.
- 1 out of 2 series of monomeric derivatives was carried out.
- All 3 series were tested *in vitro* against tumor-associated isoforms of Carbonic Anhydrases.

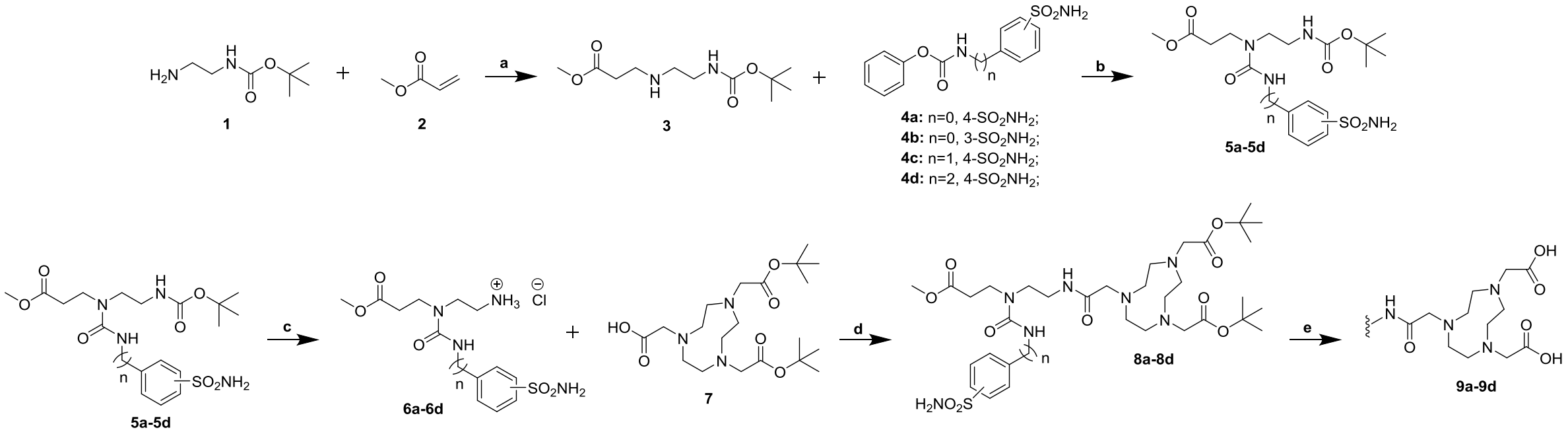






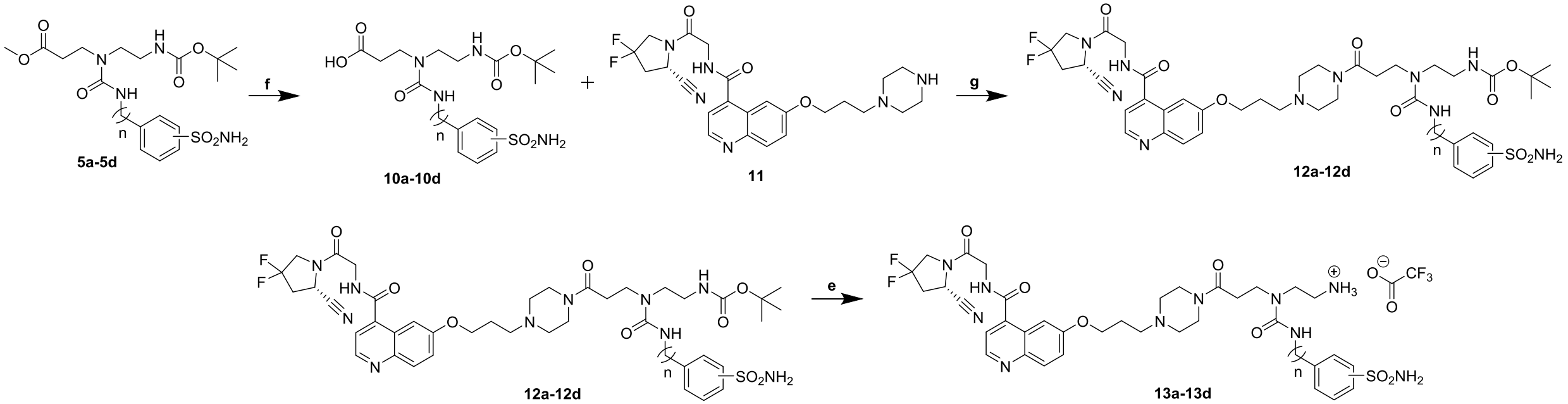


Synthesis of monomeric compounds targeting Carbonic Anhydrase bearing the radiolabeling chelator



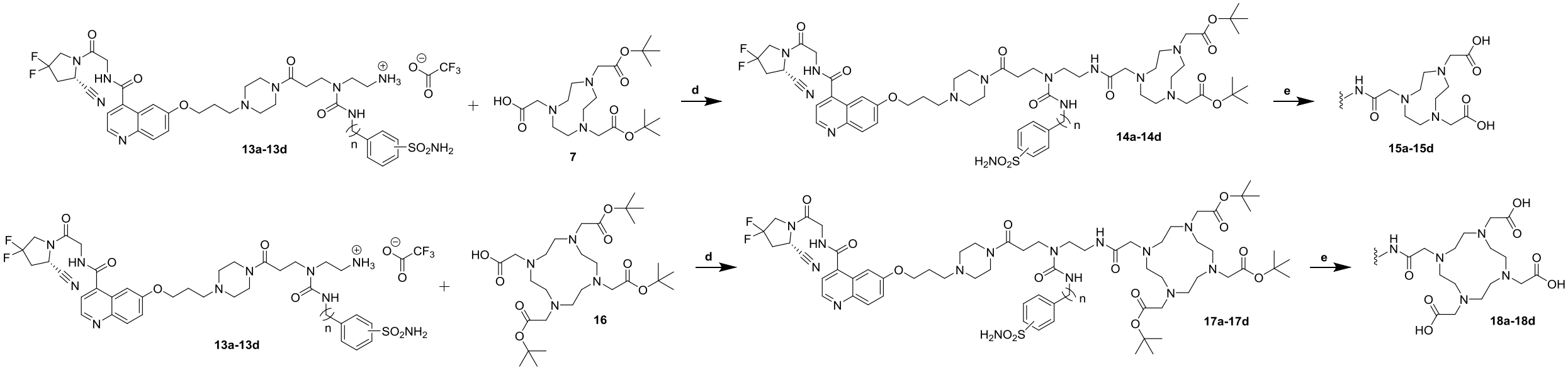
Reagents and reaction conditions: a) dry MeOH, 0 °C to r.t., o.n.; b) dry ACN, reflux, o.n.; c) HCl 1M in EtOAc, r.t., o.n.; d) HATU, DIPEA, dry DMF, r.t., o.n.; e) TFA, r.t., 20h;

Synthesis of dimeric compounds targeting both Carbonic Anhydrase and Fibroblast Activation Protein



Reagents and reaction conditions: f) LiOH, THF/EtOH/H_2O (2:2:1), r.t., o.n.; g) PyBOP, DIPEA, dry DMF, r.t., o.n.; e) TFA, r.t., 20-24h;

Synthesis of dimeric compounds introducing the radiolabeling chelator NOTA/DOTA



Reagents and reaction conditions: d) HATU, DIPEA, dry DMF, r.t., o.n.; e) TFA, r.t., 20-24h;

CA inhibition activity evaluation by means of stopped-flow CO_2 hydrase *in vitro* assay

Code	K_I (nM)[a]					
	CA I	CA II	CA VA	CA IX	CA XII	CA XIII
8a	4236.8	17.8	619.2	128.5	48.4	607.6
8b	2400.3	172.5	570.4	2700.1	50.1	180.6
8c	902.1	51.1	833.8	419.2	51.6	743.1
8d	958.5	183.3	808.3	2048.0	39.2	334.3
9a	>100000	2855.6	413.0	7410.3	59.6	188.8
9b	1301.5	82.7	820.1	315.2	25.4	290.6
9c	3829.0	43.5	512.9	1703.4	43.4	259.7
9d	1451.8	81.5	635.5	426.6	37.1	417.9
12a	2922.8	132.6	494.1	283.1	46.4	181.3
12b	4254.3	127.6	596.6	3015.7	44.7	497.4
12c	3993.2	41.4	613.3	1672.2	127.4	33.6
12d	2321.1	165.9	550.0	2643.2	45.5	170.3
13a	1269.0	76.6	809.8	306.5	24.2	287.0
13b	48719	820.1	602.4	179.1	45.4	329.2
13c	14918	134.2	681.8	869.5	41.6	1771.9
13d	4939.0	122.5	18509	650.7	39.6	432.1

Code	K_I (nM)[a]					
	CA I	CA II	CA VA	CA IX	CA XII	CA XIII
14a	10012	131.9	496.2	1099.8	31.0	276.5
14b	42992	1314.4	396.6	1830.1	44.7	654.3
14c	20451	165.4	537.7	56.2	54.1	512.7
14d	>100000	141.2	757.2	75.7	46.8	389.8
15a	2979.5	162.3	445.3	1741.4	42.5	825.0
15b	>100000	142.8	349.3	1888.6	47.6	485.2
15c	15447	135.4	610.2	3100.5	46.3	500.8
15d	2872.7	143.1	634.2	838.0	49.9	721.4
17a	305.1	547.0	697.8	397.1	56.7	530.2
17b	1197.4	921.3	621.9	552.5	261.3	678.8
17c	>100000	1579.0	429.6	5965.2	53.1	693.5
17d	3021.5	145.7	512.3	290.4	48.2	192.8
18a	384.7	825.9	427.7	357.1	61.8	250.3
18b	>100000	1434.6	711.9	4934.3	63.9	243.5
18c	>100000	1761.6	400.0	5352.9	62.2	362.1
18d	4562.3	1067.9	551.6	2964.1	52.8	473.5
AAZ	250.0	12.1	63.0	25.6	5.7	17.0

[a] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10% of the reported values)

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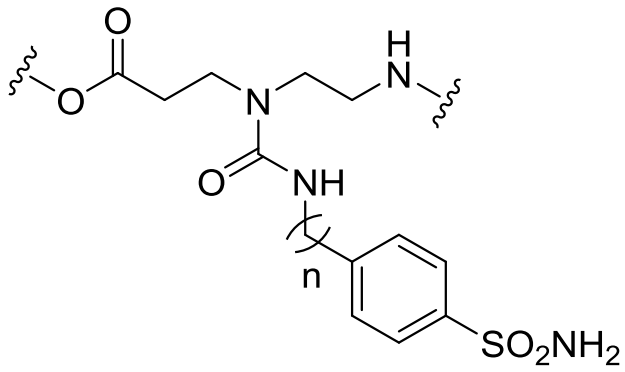

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8c	902.1	51.1	833.8	419.2	51.6	743.1	14c	20451	165.4	537.7	56.2	54.1	512.7	n=1
8d	958.5	183.3	808.3	2048.0	39.2	334.3	14d	>100000	141.2	757.2	75.7	46.8	389.8	n=2
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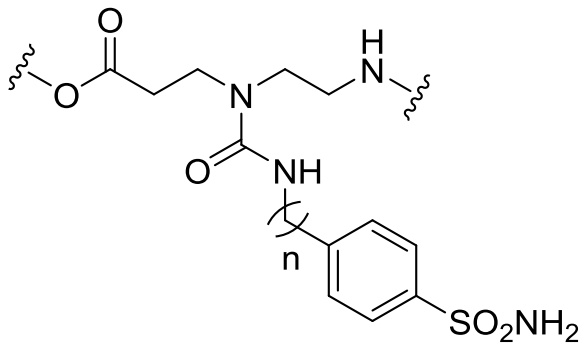

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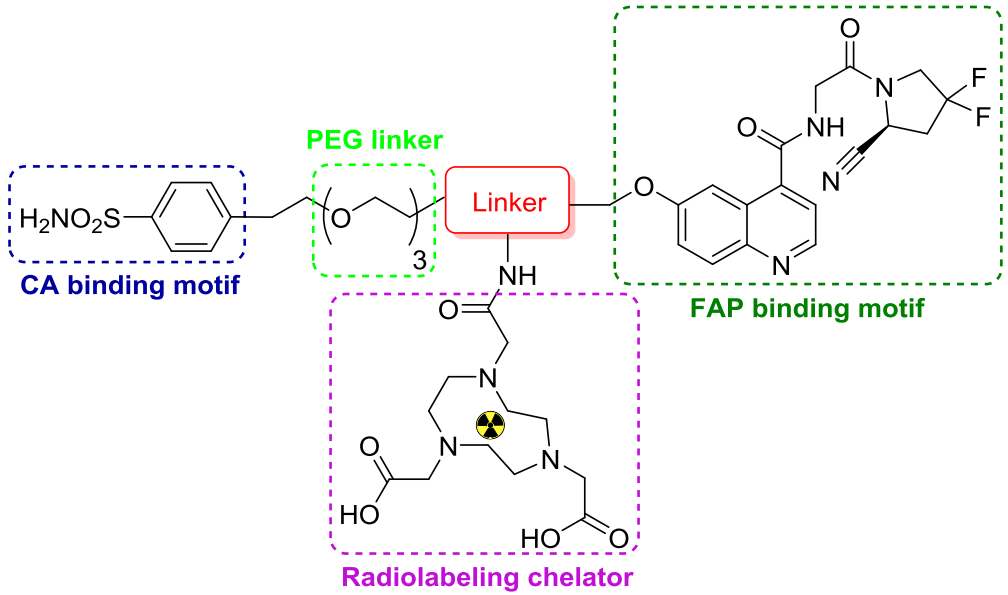
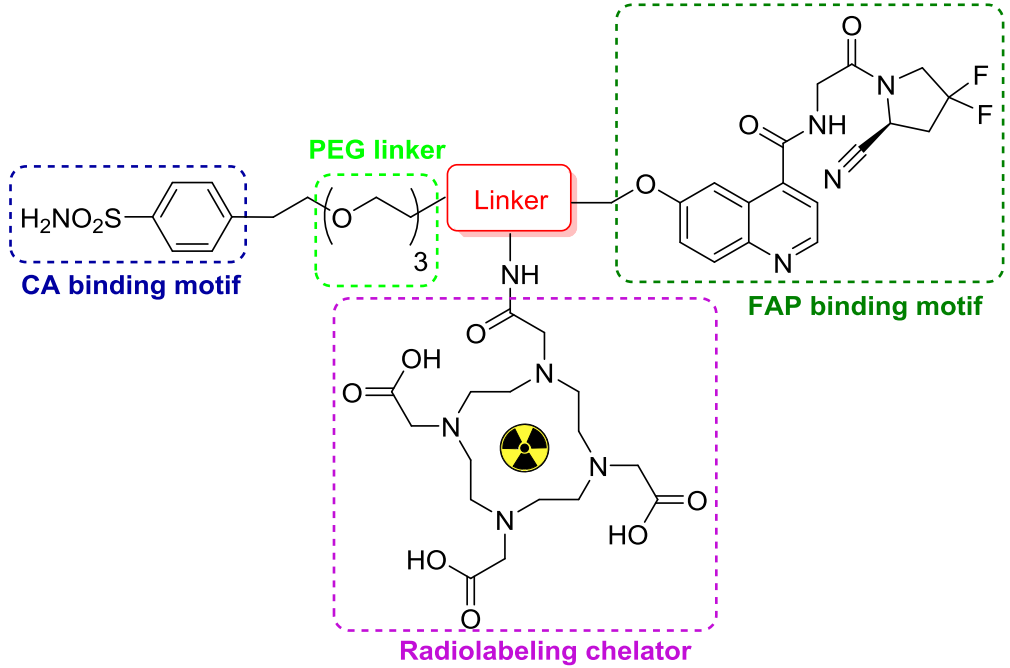
CONCLUSIONS AND FUTURE PERSPECTIVES

- 2 new series of dual inhibitors bearing the radiolabeling chelator will be synthesized by using PEG linker for increasing yields and radiolabeling efficiency.

- All synthesized derivatives will be evaluated *in vitro* against FAP-α and subsequently evaluated *in vivo*.





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Thank you for your kind attention!

12/12/2024