POTENT AND SELECTIVE INHIBITION OF HUMAN Ca^{2+}-INDEPENDENT PHOSPHOLIPASE A_2 BY FLUOROKETONES

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Introduction

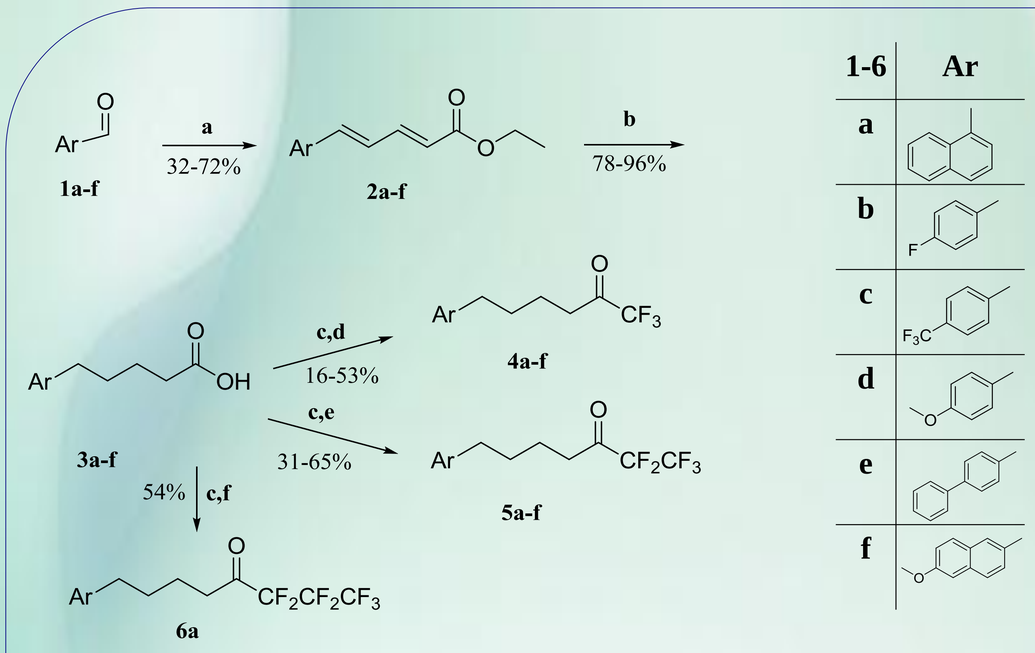
The superfamily of phospholipases A_2 (PLA_2) consists of hydrolytic enzymes that act upon the *sn-2* ester bond of phospholipids, releasing free fatty acids and lysophospholipids. The main representative of these fatty acids is arachidonic acid, which can be transformed into eicosanoids (prostaglandins, leukotriens, etc) by the action of other enzymes. Lysophospholipids are precursors for other bioactive compounds, such as platelet-activating factor (PAF). PAF and eicosanoids constitute basic mediators of inflammation. The three predominant groups of phospholipase A_2 found in human tissues are the cytosolic PLA_2 ($cPLA_2$), the calcium-independent PLA_2 ($iPLA_2$) and the secreted PLA_2 ($sPLA_2$). $iPLA_2$ has been proposed as a homeostatic enzyme involved in basal metabolism within the cell, but has also been found to be involved in diseases of the brain, the heart and the central nervous system, which makes this enzyme an attractive drug target. We have recently demonstrated that Ca^{2+}-independent phospholipase A_2 (GVIA $iPLA_2$) plays a key-role in experimental autoimmune encephalomyelitis and that GVIA $iPLA_2$ is a novel target for the development of new therapies for multiple sclerosis.[1]

A series of fluoroketones has been presented as $iPLA_2$ inhibitors and the structure-activity relationship has been evaluated.[2] To extend this research, we synthesized a variety of polyfluoroketones containing an aromatic ring and a four carbon atom chain between the ring and the polyfluoroketone group.

Synthesis

Commercially available aromatic aldehydes **1a-f** were used as starting materials, to prepare the desired fluoroketones **(4a-f, 5a-f, 6a)**. Each aldehyde underwent a Horner – Wadsworth – Emmons reaction with triethyl-4-phosphonocrotonate in the presence of LiOH to produce the corresponding unsaturated ester **2a-f**. After catalytic hydrogenation and saponification with 1 N NaOH in ethanol we acquired the corresponding carboxylic acids **3a-f** which were converted to acyl chlorides by the oxalyl chloride/DMF method. In situ, acyl chlorides were treated with pyridine and trifluoroacetic anhydride or pentafluoropropionic anhydride or heptafluorobutanoic anhydride to provide trifluoromethyl ketones **4a-f,** pentafluoroethyl ketones **5a-f** and the heptafluoropropyl ketone **6a**, respectively **(Scheme 1)**.

Scheme 1: Synthesis of polyfluoroketones



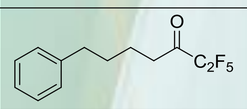
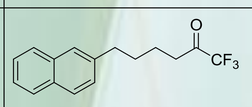
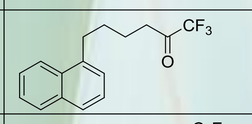
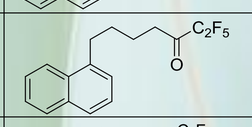
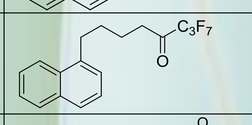
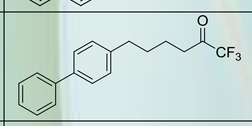
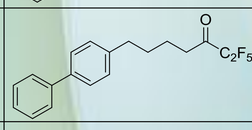
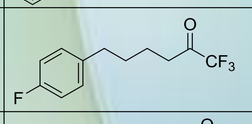
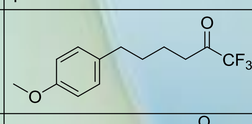
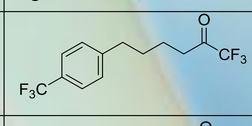
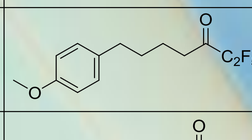
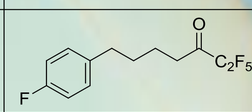
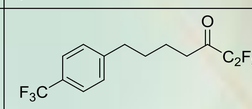
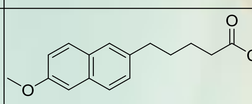
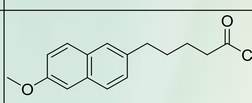
1-6	Ar
a	
b	
c	
d	
e	
f	

Reagents and conditions: a) $C_2H_5OOCCH{=}CHCH_2P(=O)(OC_2H_5)_2$, LiOH, THF, 77 °C, b) i) H_2, 10% Pd/C, EtOH, ii) NaOH 1N, EtOH, c) $(COCl)_2$, DMF, CH_2Cl_2, d) pyridine, $(CF_3CO)_2O$, CH_2Cl_2, 0 °C to r.t., e) pyridine, $(CF_3CF_2CO)_2O$, CH_2Cl_2, 0 °C to r.t., f) pyridine, $(CF_3CF_2CF_2CO)_2O$, CH_2Cl_2, 0 °C to r.t.

In vitro results

All synthesized compounds were tested for inhibition on human GVIA $iPLA_2$, GIVA $cPLA_2$ and GV $sPLA_2$. **(Table 1)** The percentage of inhibition for each enzyme was determined at 0.091 mol fraction, and $X_I(50)$ values were measured for inhibitors which showed more than 93% inhibition for an enzyme. When compared to the known inhibitor **FKGK18**, changing the position of the carbon chain on the naphthalene ring, leads to less potency, but higher selectivity. Increasing the number of fluorine atoms, **GK172** and **GK173** become less potent inhibitors of $iPLA_2$. When a methoxy group is added to the naphthalene ring, inhibitors **GK213** and **GK214** lose selectivity towards $iPLA_2$. The biphenyl group instead of the naphthalene shows a similar inhibition for both $iPLA_2$ and $cPLA_2$ **(GK174, GK175)**. The insertion of a trifluoromethyl group on the aromatic ring presented excellent potency (**GK178** and **GK189**), but poor selectivity. In the case of **GK176** and **GK188** the fluorine on the aromatic ring shows both great potency and selectivity towards $iPLA_2$. When the fluorine atom is replaced by a methoxy group, compounds **GK177** and **GK187** are currently the most potent and selective inhibitors for $iPLA_2$. Inhibitors **GK176**, **GK177**, **GK187** and **GK188** can be used to study the role of GVIA $iPLA_2$ in neurological disorders.

Table 1: Inhibition of PLA_2 by fluoroketones

No	Structure	$iPLA_2$		$cPLA_2$		$sPLA_2$
		% inhibition	$X_I(50)$	% inhibition	$X_I(50)$	% inhibition
FKGK11		99.4	0.0014 ± 0.0001	N.D.		28
FKGK18		99.9	0.0002 ± 0.0000	93.1	0.039 ± 0.001	36.8
GK171, 4a		98.7	0.0018 ± 0.0002	77.4		31.9
GK172, 5a		96.4	0.0034 ± 0.0002	64.0		29.4
GK173, 6a		76.7		60.4		57.8
GK174, 4e		97.9	0.0189 ± 0.0045	96.5	0.0074 ± 0.0003	40.5
GK175, 5e		94.6	0.0134 ± 0.0017	72.6		38.3
GK176, 4b		98.5	0.0002 ± 0.0000	41.4		N.D.
GK177, 4d		99.8	0.0001 ± 0.0000	54.3		N.D.
GK178, 4c		99.9	0.0002 ± 0.0000	83.9		30.4
GK187, 5d		99.8	0.0001 ± 0.0000	12.9		32.8
GK188, 5b		98.2	0.0003 ± 0.0000	22.2		29.3
GK189, 5c		98.9	0.0003 ± 0.0001	62.5		36.9
GK213, 4f		92.6		85.5		41.7
GK214, 5f		92.5		54.7		63.1

N.D. signifies compounds with less than 25% inhibition (or no detectable inhibition).

References

1.Kalyvas, A.; Baskakis, C.; Magrioti, V.; Constantinou-Kokotou, V.; Stephens, D.; Dennis, E. A.; Kokotos G.; David, S. *Brain* **2009**, *132*, 1221-1235

2. Kokotos, G.; Hsu, Y.-H.; Burke, J. E.; Baskakis, C.; Kokotos, C.G.; Magrioti, V.; Dennis, E. A. *J. Med. Chem.* **2010**, *53*, 3602-361.

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