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Pyridazin-3(2H)-one as new FABP4 inhibitors suggested by molecular growing experiments

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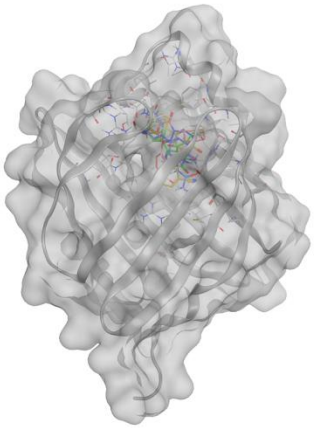
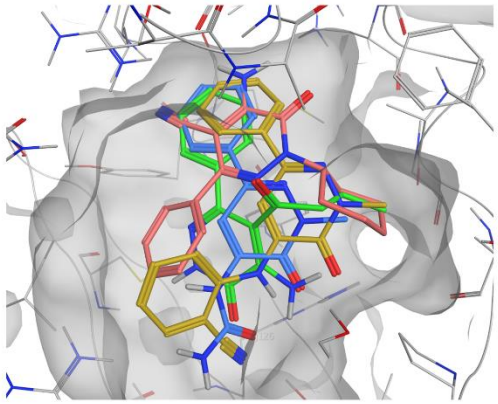
Pyridazin-3(2H)-one as new FABP4 inhibitors suggested by molecular growing experiments



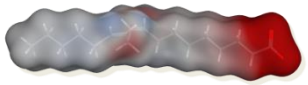


FABP4 inhibition	IC_{50} (μM)
Arachidonic acid	3.42 ± 0.54
4b	8.27 ± 0.20
25a	2.97 ± 0.26
30b	23.18 ± 0.52
22	15.23 ± 0.76

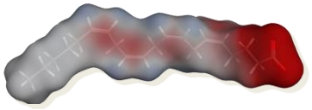
Abstract: The therapeutic potential of fatty acid binding protein 4 (FABP4) is widely acknowledged. Currently, there are numerous clinical studies that indicate how fatty acid binding protein 4 inhibitors could be useful in the treatment of various diseases. To identify new and more potent inhibitors, we utilized a two-step computational approach to design novel structures. Through the use of this approach, we were able to identify a new class of FABP4 inhibitors (FABP4i IC_{50} 2.97 to 23.18 μM) that are capable of inhibiting the activity of the protein as low as Arachidonic acid (FABP4i IC_{50} 3.42 ± 0.54 μM). In this communication, we present the detailed structural, biological evaluation as well as the synthetic procedures of the new pyridazinone-based scaffold FABP4i.

Keywords: Fatty acid binding protein; FABP4; FABP4is; FABP4 inhibitors; pyridazinone; computing assisted molecular design

Introduction



Linoleic acid



Arachidonic acid

Chronically elevated plasma fatty acid leads to pathophysiological disorders:

- Diabetes
- Obesity
- Atherosclerosis

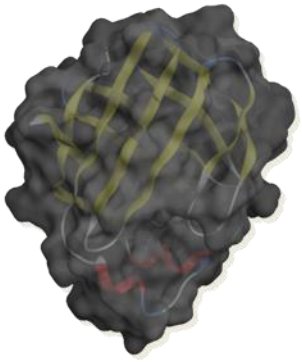
FAs are insoluble in water, and their trafficking into the body requires specific carriers such as the fatty acid-binding proteins (FABPs) family.

WHY DO WE WANT TO INHIBIT THIS PROTEIN?

Metabolic syndrome

FABP4 (aP2 or A-FABP) is the one expressed in adipocytes and the research into small molecules inhibitors for such protein initially started when it was reported that knockout animal models of FABP4 produced protective effects against the development of insulin resistance, as well as several pathological events linked to the metabolic syndrome and atherosclerosis

Introduction



1. L-FABP (liver)
2. I-FABP (intestinal)
3. H-FABP (muscle and heart)
4. A-FABP (adipocyte)
5. E-FABP (epidermal)
6. Il-FABP (ileal)
7. B-FABP (brain)
8. M-FABP (myelin)
9. T-FABP (testis)

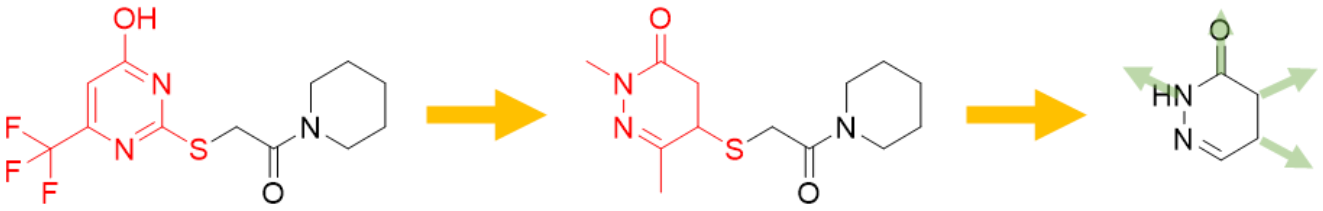
Modified FABPs expression patterns were described for prostate, bladder, renal cell carcinoma and other types of cancer cells:

- **Promotion of methastasis** (ovarian cancer)
- **Promotes cancer cell progression** (prostate)
- Biomarker

Non-physiological expression of FABPs are present in some of the most common cancers such as renal cell carcinoma, bladder and prostate, as well as other types of cancer cells

Results and discussion

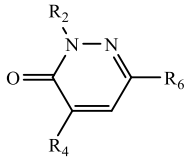
To generate a novel series of FABP4 inhibitors we have exploited a two-step computing assisted molecular design. As showed below, in the first step of the drug-design process we focused on the search for bioisosteric replacements/scaffold-hopping of the pyrimidine scaffold of the co-crystallyzed ligand (2-[(2-oxo-2-piperidin-1-ylethyl)sulfanyl]-6-(trifluoromethyl)pyrimidin-4-ol) pdbID: 1TOU. Our bioisosteric replacement analysis led to the selection of three nitrogen containing heterocyclic frameworks, i.e. pyridazinones, pyridines and benzo[d]thiazole. Considering the synthetic accessibility of pyridazinone-based molecules and that pyridazinone was not investigated earlier as a scaffold to access FABP4 inhibitors, we envisaged to use this heterocycle to carry out automated ligand growing experiments inside the FABP4 cavity leading to 52 target molecules

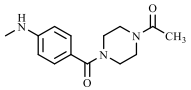


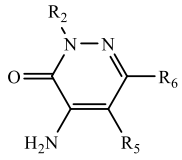
Results and discussion

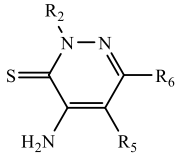
FABP4 inhibitory activity was assessed by measuring the decrease in fluorescence of a detection reagent when displaced by a strong ligand of FABP4. The series of synthesized molecules were screened in a two-step procedure. Firstly, a single concentration of 5 μM was used for all of the molecules and then only compounds that were able to reduce the fluorescence reading (at least 95%) were fully evaluated and the IC_{50} (μM) measured and compared with the one of the potent ligand arachidonic acid. The single point displacement results are reported in the following slide. Considering the results of the first screening, 10 molecules were selected as best compounds able to reduce the fluorescence of the DR to at least 95% and the IC_{50} (μM) was calculated. Arachidonic acid, a known powerful ligand of FABP4, was used as a positive control and revealed an IC_{50} of 3.42 μM. IC_{50} of our set of compounds are reported in **Table 3** and **25a** showed the best result showed with IC_{50} of 2.97 μM.

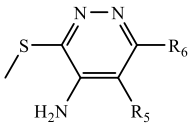
Results and discussion



Comp.	R_2	R_4	R_6
25a	CH_3	$NHCONH_2$	Ph
25b	cC_6H_{11}	$NHCONH_2$	Ph
25c	C_2H_5	$NHCONH_2$	Ph
25d	iC_3H_7	$NHCONH_2$	Ph
25e	nC_3H_7	$NHCONH_2$	Ph
25f	nC_4H_9	$NHCONH_2$	Ph
28	H	$NHCONH_2$	Ph
29a	CH_3	$NHCOCH_3$	Ph
29b	CH_3	$NHCOC_2H_5$	Ph
29c	CH_3	$NHCOiC_3H_7$	Ph
29d	CH_3	$NHCOnC_3H_7$	Ph
30a	CH_3	NH-(3-CN)-Ph	Ph
30b	CH_3	NH-(2-CN)-Ph	Ph
31a	CH_3	NH-(3-$CONH_2$)-Ph	Ph
31b	CH_3	NH-(2-$CONH_2$)-Ph	Ph
35	CH_3		Ph
39a	CH_3	$NHCONH_2$	cC_6H_{11}
39b	CH_3	$NHCONH_2$	iC_3H_7
40	H	$NHCONH_2$	2-(OH)-Ph
43	CH_3	$NHCONH_2$	2-(OH)-Ph
49	CH_3	$NHCONH_2$	2-pyridinyl
51	CH_3	$CONH_2$	Ph
55	Ph	$NHCONH_2$	CH_3

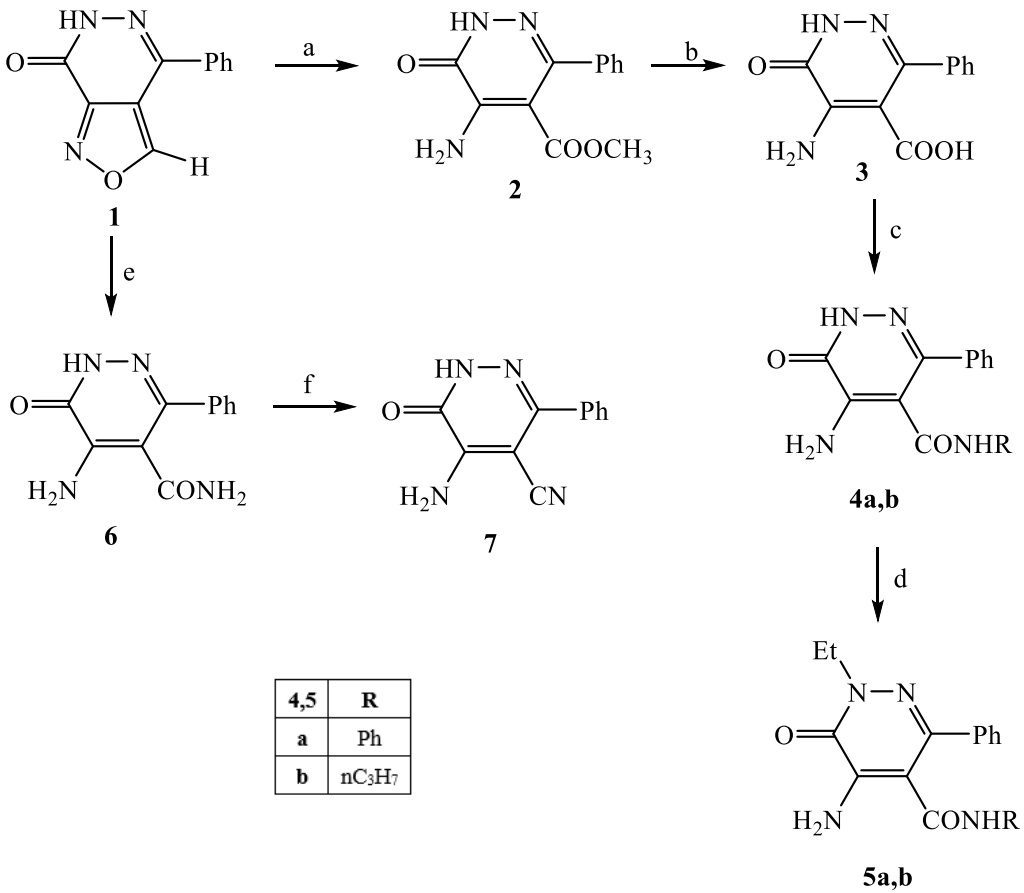






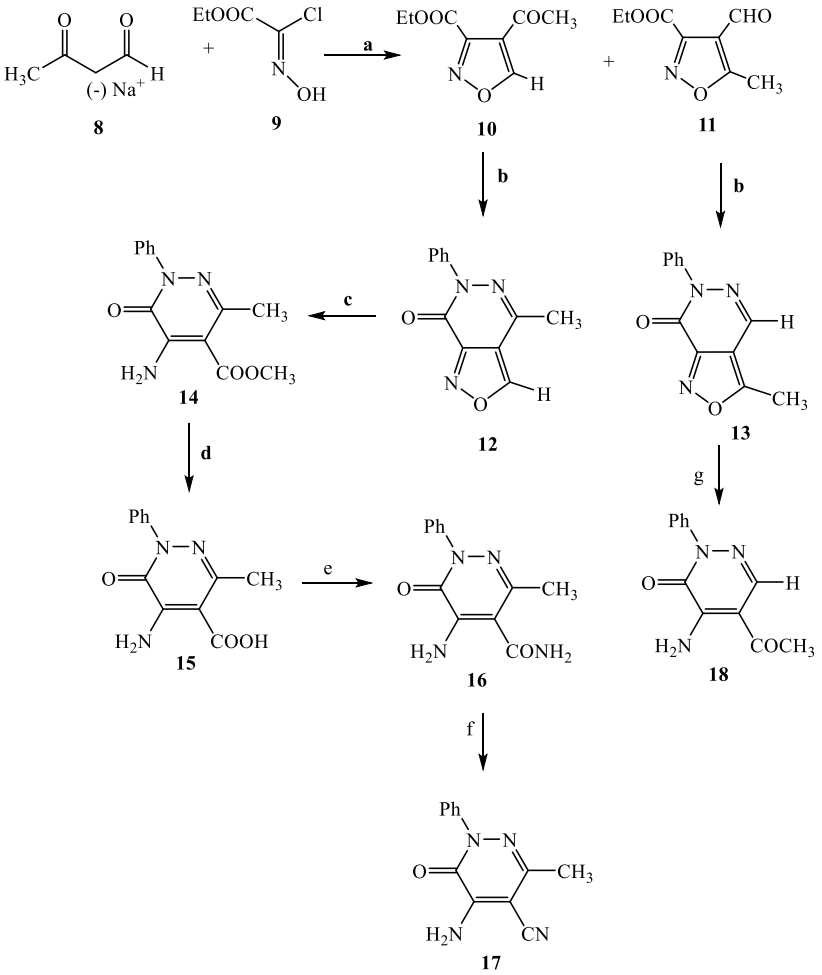
32 **27**

Comp.	R_2	R_5	R_6
4a	H	CONHPh	Ph
4b	H	$CONHnC_3H_7$	Ph
5a	C_2H_5	CONHPh	Ph
5b	C_2H_5	$CONHnC_3H_7$	Ph
6	H	$CONH_2$	Ph
7	H	CN	Ph
16	Ph	$CONH_2$	CH_3
17	Ph	CN	CH_3
18	Ph	$COCH_3$	H
21	cC_6H_{11}	$CONH_2$	Ph
22	cC_6H_{11}	CN	Ph
24d	iC_3H_7	H	Ph
24e	nC_3H_7	H	Ph
24f	nC_4H_9	H	Ph
27	-	H	Ph
32	CH_3	H	Ph
37a	H	H	3-thienyl
37c	H	H	cC_6H_{11}
37d	H	H	iC_3H_7
38a	CH_3	H	3-thienyl
38b	CH_3	H	cC_6H_{11}
38c	CH_3	H	iC_3H_7
38d	CH_3	H	CH_2-Ph
42a	CH_3	H	2-(OH)-Ph
42b	CH_3	H	4-(NH_2)-Ph
44	CH_3	H	4-($NHCOCH_3$)-Ph
48	CH_3	H	2-pyridinyl
54	Ph	H	CH_3
57	Ph	pyrazole	CH_3

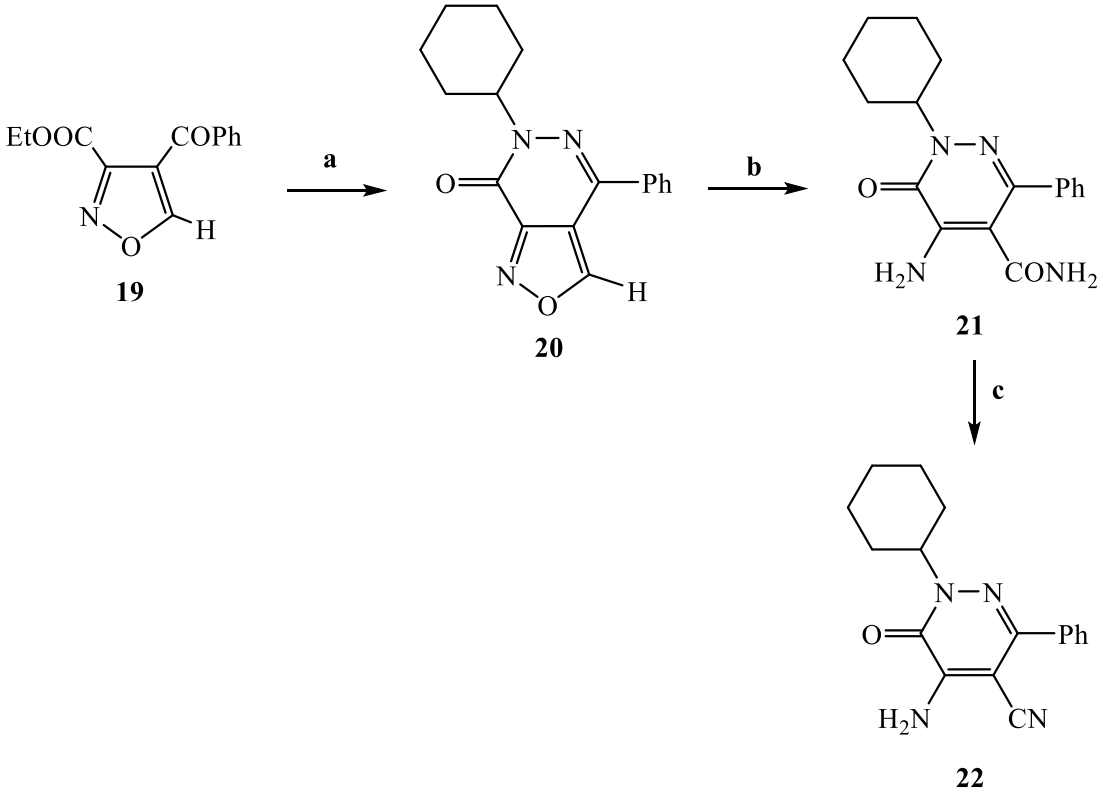


4,5	R
a	Ph
b	nC_3H_7

[a]**Reagents and conditions: (a)** Et_3N, CH_3OH, 60 °C, 2 h; **(b)** 6N NaOH, EtOH, reflux, 30 min; **(c)** (i) $SOCl_2$, Et_3N, r.t., 30 min; (ii) R-NH_2, anhydrous THF, r.t., 2 h; **(d)** CH_3CH_2Br, K_2CO_3, anhydrous DMF, reflux, 30-90 min; **(e)** 33% NH_4OH, $C_5H_{11}N$, 60 °C, 90 min; **(f)** $POCl_3$, 60 °C, 2h.

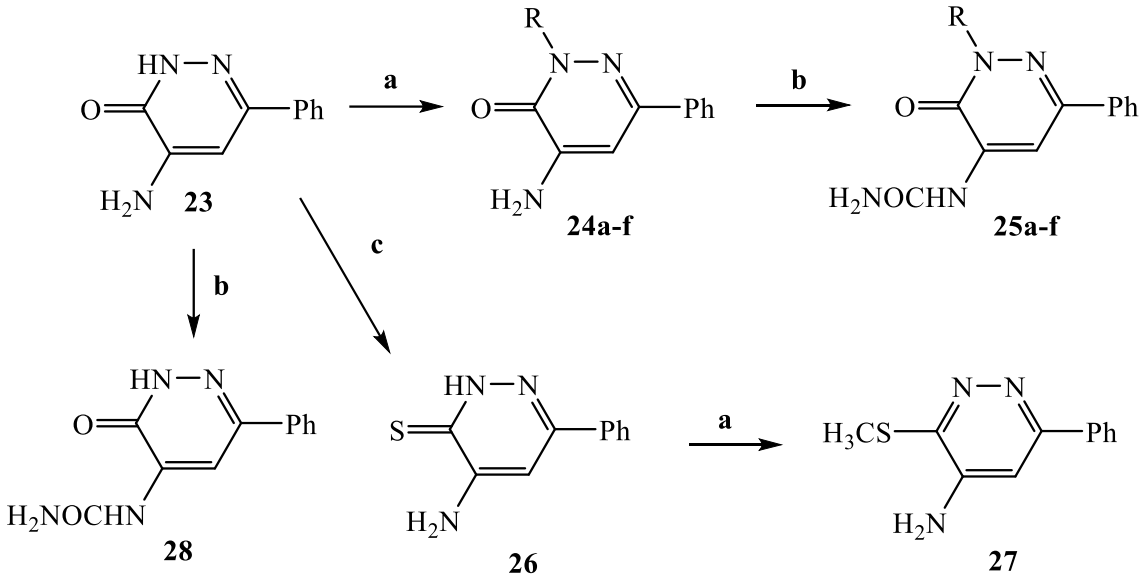


[a]**Reagents and conditions: (a)** anhydrous EtOH, 0 °C, 1h; **(b)** phenylhydrazine, PPA, EtOH, 70 °C, 30 min; **(c)** CH_3OH, Et_3N, 60 °C, 2h; **(d)** NaOH, EtOH, reflux, 30 min; **(e)** (i) $SOCl_2$, Et_3N, reflux, 30 min.; (ii) 33% NH_4OH, anhydrous THF, r.t., 15 min; **(f)** $POCl_3$, 60 °C, 2h; **(g)** $HCOONH_4$, 10% Pd/C, EtOH, reflux, 2 h.

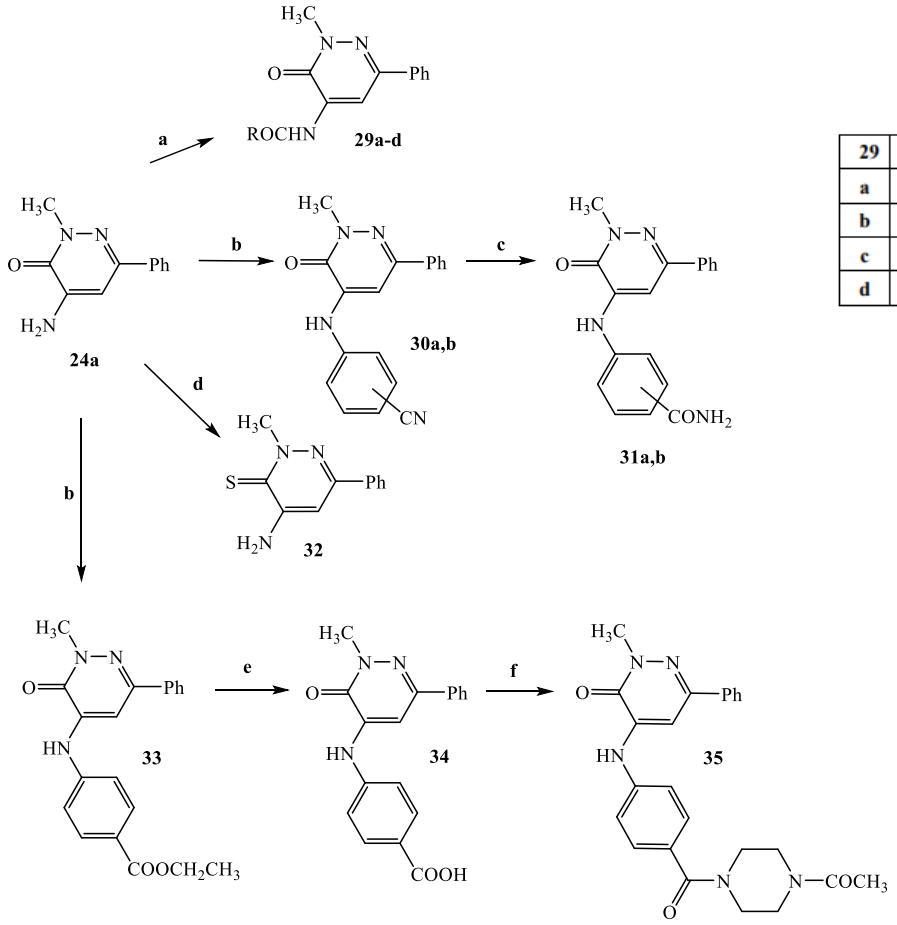


[a]**Reagents and conditions: (a)** cyclohexylhydrazine, PPA, EtOH, 70 °C, 30 min; **(b)** 33% NH_3, piperidine, 60 °C, 90 min; **(c)** $POCl_3$, 60 °C, 2h.

24,25	R
a	CH_3
b	cC_6H_{11}
c	C_2H_5
d	iC_3H_7
e	nC_3H_7
f	C_4H_9



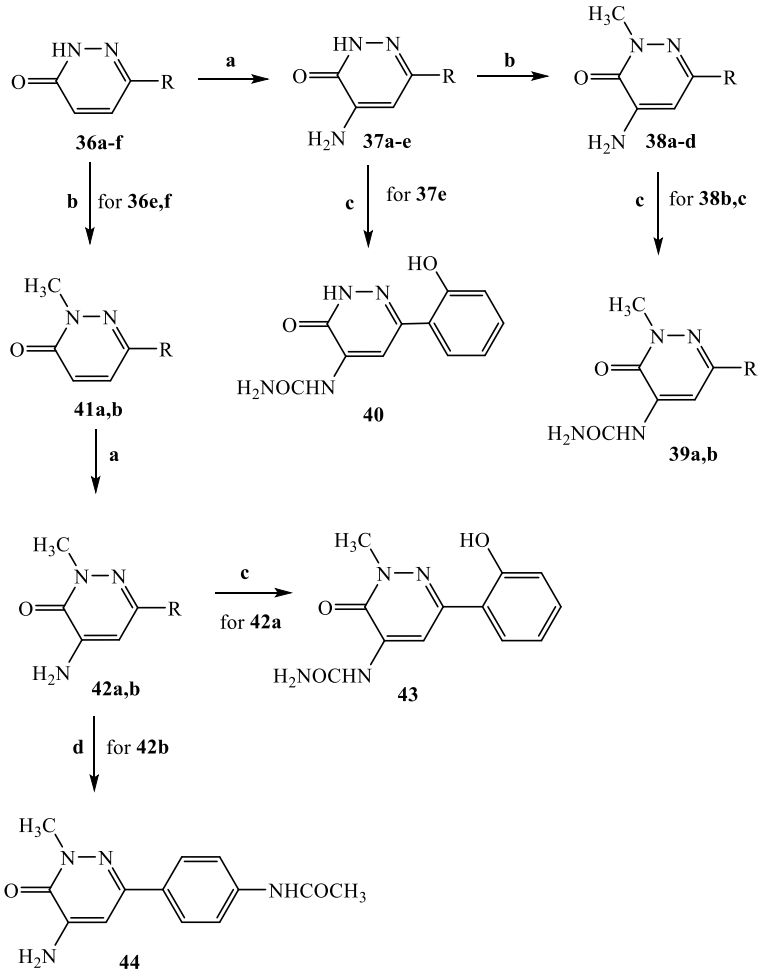
[a]**Reagents and conditions: (a)** suitable R-Br, K_2CO_3, anhydrous DMF, reflux, 1-4 h; **(b)** (i) dry THF, CH_3COONa, 0°C then triphosgene, reflux, 2 h; (ii) NH_3 33%, 0 °C, 1 h; **(c)** Lawesson's reagent, anhydrous toluene, reflux, 5 h.



29	R
a	CH_3
b	C_2H_5
c	iC_3H_7
d	nC_3H_7

Comp.	CN/CONH$_2$
30a	meta
30b	ortho
31a	meta
31b	ortho

[a]**Reagents and conditions: (a)** suitable (R-CO_2)O, anhydrous C_6H_5N, closed tube, 140 °C, 5 h; **(b)** 2/3-cyanophenylboronic acid (for **30a,b**) or 4-ethoxycarbonylphenylboronic acid (for **33**), $Cu(Ac)_2$, Et_3N, dry CH_2Cl_2, r.t., 12 h; **(c)** H_2SO_4 80%, 80 °C, 4 h; **(d)** Lawesson's reagent, anhydrous toluene, reflux, 10 h; **(e)** NaOH 6N, EtOH 96%, reflux, 1 h; **(f)** (i) $SOCl_2$, Et_3N (catalytic), reflux, 1 h; (ii) anhydrous THF, 1-acetylpiperazine, 0 °C then r.t., 1 h.



39	R
a	cC$_6$H$_{11}$
b	iC$_3$H$_7$

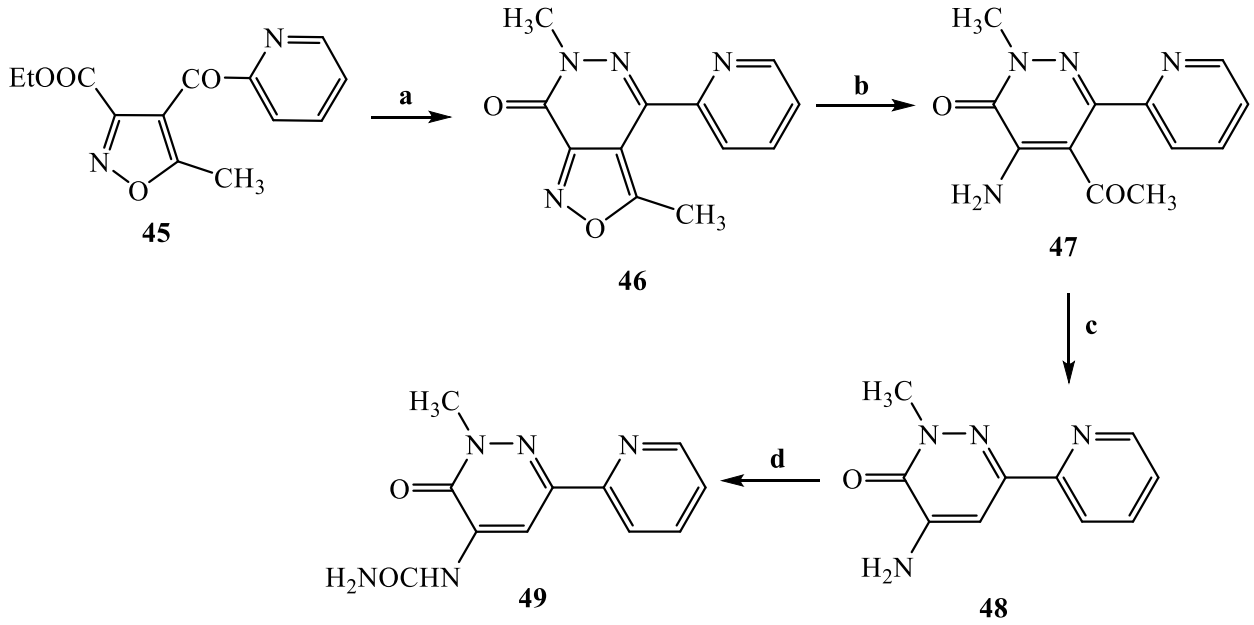
Comp.	R
41a	2-OH-Ph
41b	4-NO$_2$-Ph
42a	2-OH-Ph
42b	4-NH$_2$-Ph

36	R
a	3-thienyl
b	cC$_6$H$_{11}$
c	iC$_3$H$_7$
d	CH$_2$Ph
e	2-OH-Ph
f	4-NO$_2$-Ph

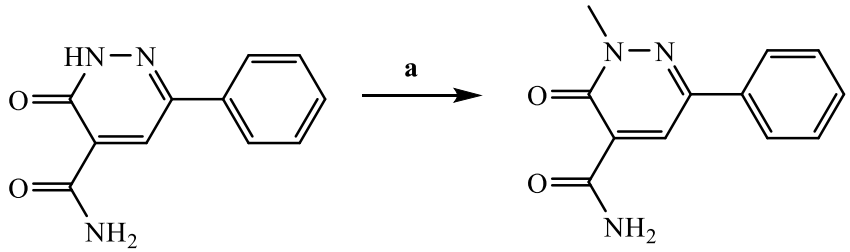
37	R
a	3-thienyl
b	cC$_6$H$_{11}$
c	iC$_3$H$_7$
d	CH$_2$Ph
e	2-OH-Ph

38	R
a	3-thienyl
b	cC$_6$H$_{11}$
c	iC$_3$H$_7$
d	CH$_2$Ph

[a]**Reagents and conditions: (a)** $NH_2NH_4 \cdot H_2O$, sealed/pressure vessel, 180 °C, 12 h; **(b)** CH_3I, K_2CO_3, anhydrous DMF, 80 °C, 2-4 h; **(c)** (i) anhydrous THF, CH_3COONa, 0°C then triphosgene, reflux, 2 h; (ii) NH_4OH 33%, 0 °C, 1 h; **(d)** $ClCOCH_3$, anhydrous THF, 0 °C, then r.t., 20 min

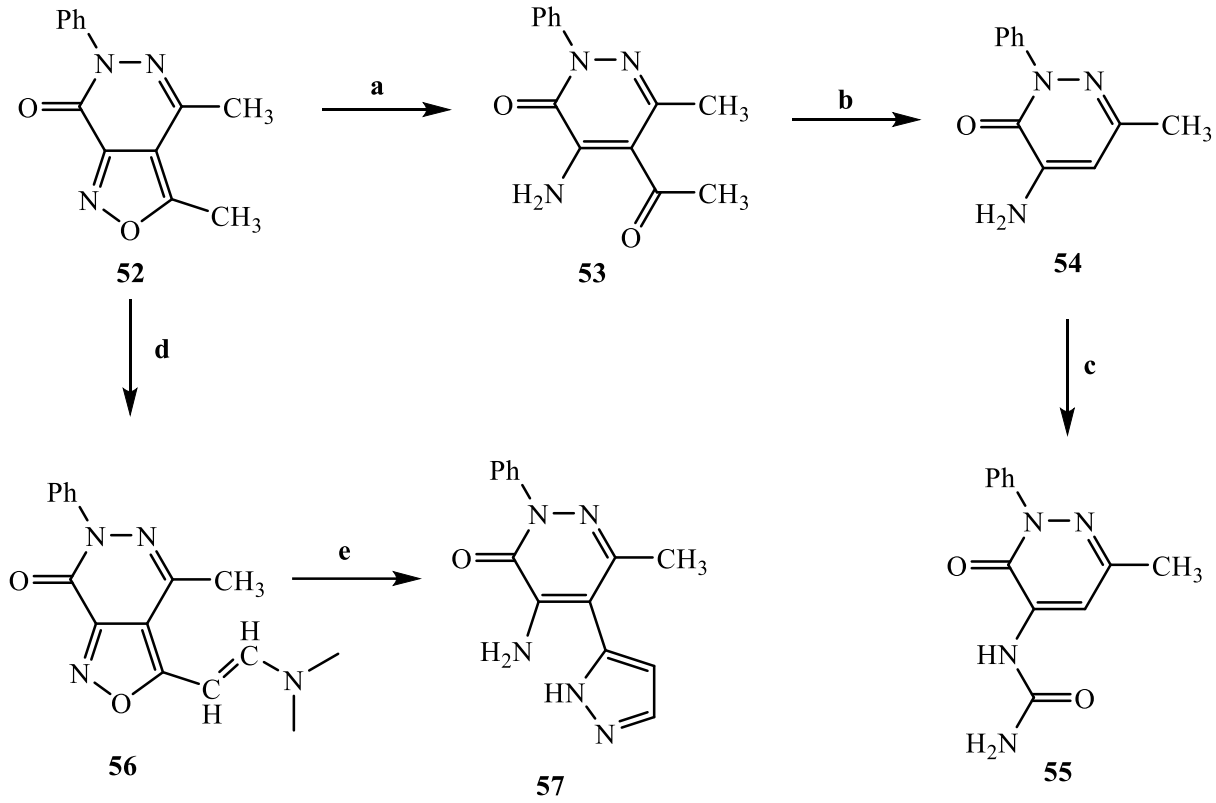


[a]**Reagents and conditions: (a)** $CH_3(NH)NH_2$, EtOH 96%, r.t., 2 h; **(b)** $HCOONH_4$, Pd/C, EtOH 96%, reflux, 2h; **(c)** HBr 48%, sealed/pressure vessel, 130 °C, 3 h; **(d)** (i) anhydrous THF, CH_3COONa, 0°C then triphosgene, reflux, 2 h; (ii) 33% NH_4OH, 0 °C, 1 h.



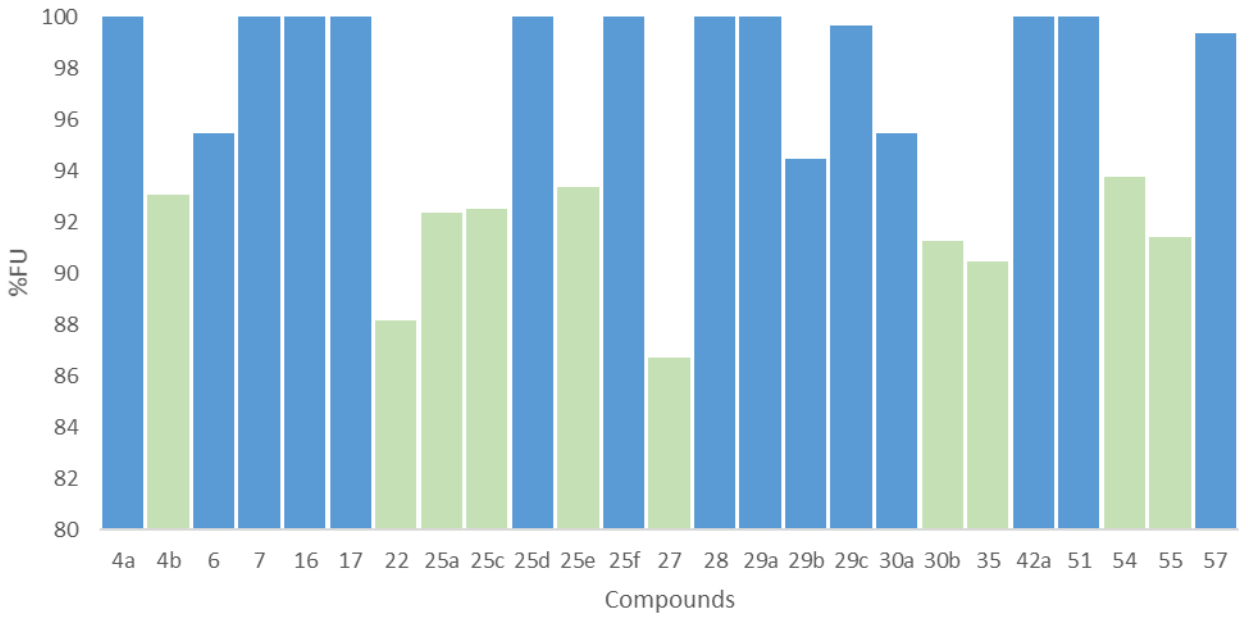
50 **51**

[a]**Reagents and conditions: (a)** CH_3I, K_2CO_3, anhydrous DMF, 80 °C, 2 h.



[a]**Reagents and conditions: (a)** $HCOONH_4$, Pd/C 10%, EtOH 96%, reflux, 2 h; **(b)** HBr 48%, sealed/pressure vessel, 130 °C, 3 h; **(c)** (i) anhydrous THF, CH_3COONa, 0°C then triphosgene, reflux, 2 h; (ii) NH_4OH 33%, 0 °C, 1 h; **(d)** DMF-DMA, 90 °C, 1 h; **(e)** $NH_2NH_4 \cdot H_2O$, anhydrous EtOH, 70 °C, 10 h.

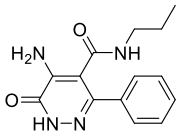
Results and discussion



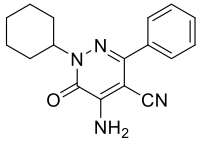
Single point displacement experiment for selected compounds

Results and discussion

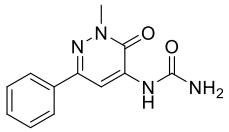
Measured IC_{50} values for selected compounds



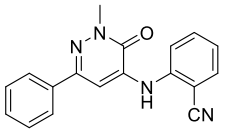
4b



22



25a

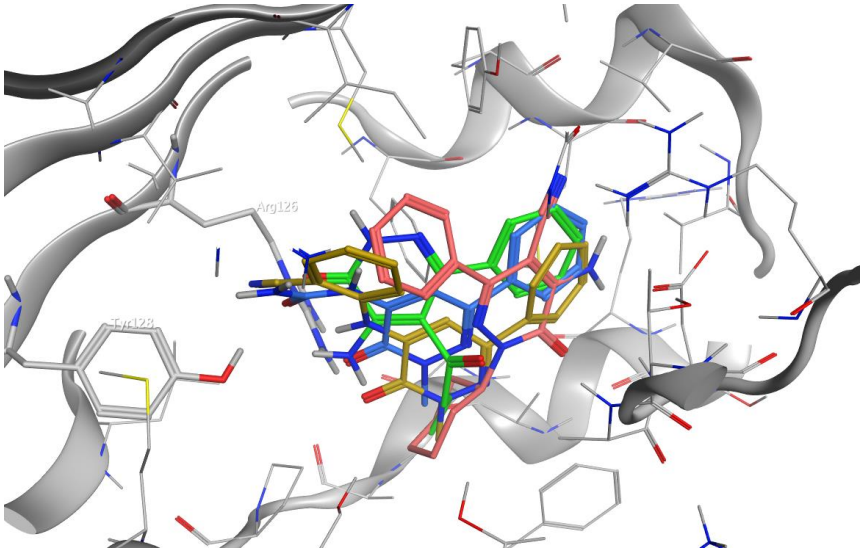


30b

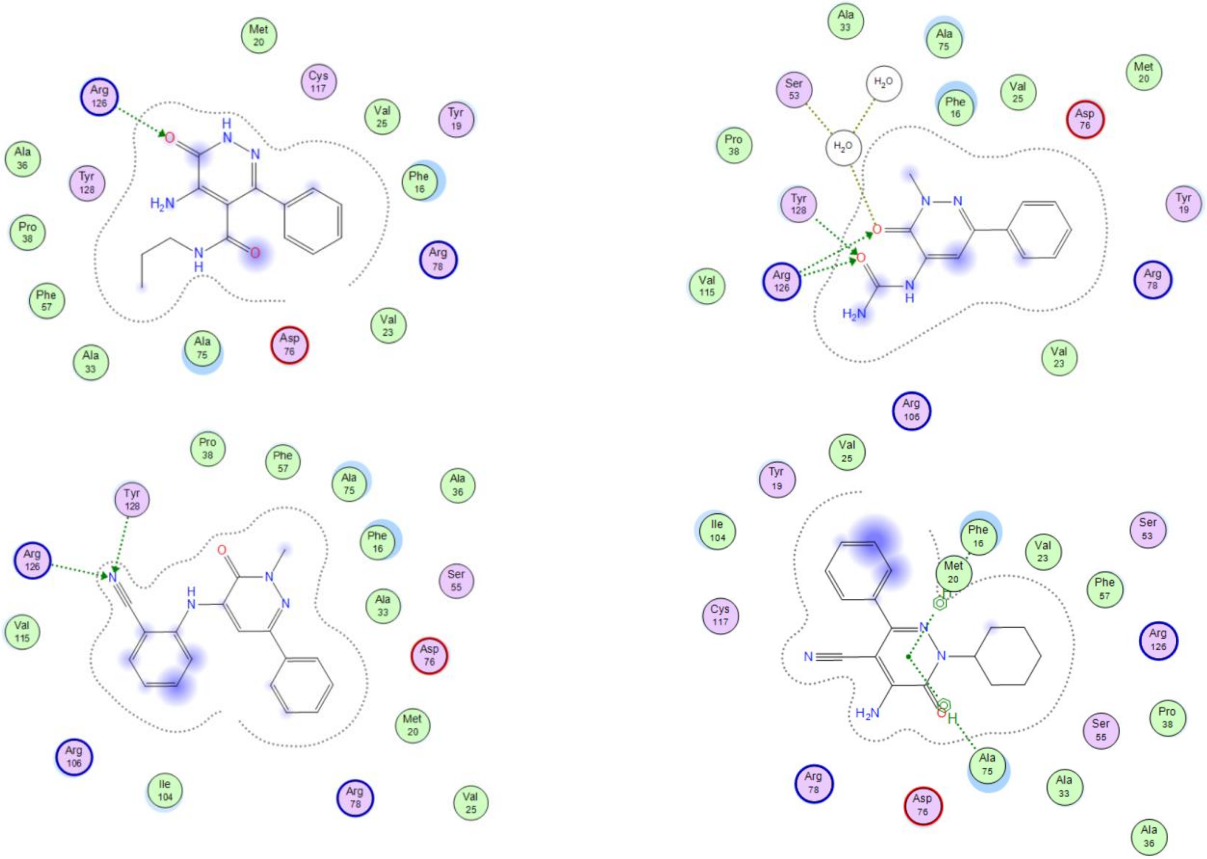
Compounds	IC_{50} (μM)
Arachidonic acid	3.42 ± 0.54
4b	8.27 ± 0.20
25a	2.97 ± 0.26
30b	23.18 ± 0.52
22	15.23 ± 0.76
25c	>50
35	>50
25e	>50
54	>50
55	>50
27	>50

Results and discussion

The docking experiments of the studied compounds were conducted on the most active compounds **4b**, **25a**, **30b**, and **22**. **Figure 3** shows the 2D binding interactions for the studied molecules while **Figure 4** displays the predicted poses inside the binding pocket of FABP4. All the molecules are able to engage several interactions with relevant residues in the binding pocket



Results and discussion



Up left) 2D interaction between **4b** and FABP4. Up right) 2D interaction between **25a** and FABP4. Down left) 2D interaction between **30b** and FABP4. Down right) 2D interaction between **22** and FABP4.

Conclusions

We have identified novel pyridazinone-based FABP4 inhibitors whose design was directed by computing assisted molecular design

Several compounds have been synthesized and tested for their ability to inhibit FABP4.

Among the new series, ten compounds were firstly selected for their inhibitory activity over FABP4 using a single point displacement assay.

In particular, **4b**, **25a**, **30b** and **22** exhibited high FABP4 inhibitory activity with IC_{50} in the low micromolar range.

The results showed that compound **25a** was the most active in terms of displacement of the arachidonic acid showing IC_{50} of 2.97 μM, which is higher than the positive control, docking experiments confirmed the interaction with the binding pocket of the protein