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

Chaired by **DR. ALFREDO BERZAL-HERRANZ**;
Co-Chaired by **PROF. DR. MARIA EMÍLIA SOUSA**



pharmaceuticals



Giuseppe Floresta ^{1,*}, Letizia Crocetti ², Chiara Zagni ¹ and Agostino Cilibrizzi ^{3,*}

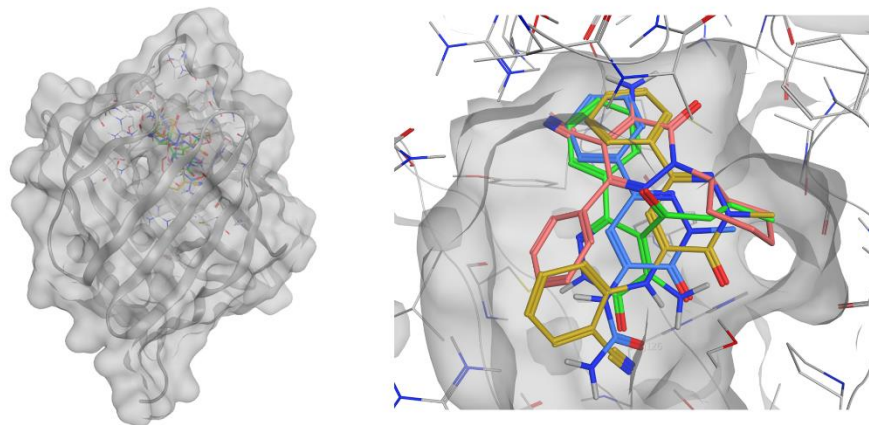
¹ Dipartimento di Scienze del Farmaco e della Salute, Università di Catania

² Dipartimento NEUROFARBA – Pharmaceutical and Nutraceutical Section

³ King's College London, Institute of Pharmaceutical Science

* Corresponding author: giuseppe.floresta@unict.it, agostino.cilibrizzi@kcl.ac.uk

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

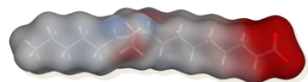


| FABP4 inhibition | IC ₅₀ (μM) |
|------------------|-----------------------|
| Arachidonic acid | 3.42 ± 0.54 |
| 4b | 8.27 ± 0.20 |
| 25a | 2.97 ± 0.26 |
| 30b | 23.18 ± 0.52 |
| 22 | 15.23 ± 0.76 |

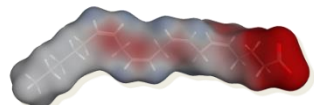
Abstract: The therapeutic potential of fatty acid binding protein 4 (FABP4) is widely acknowledged. Currently, there are numerous clinical studies that indicate how fatty acid binding protein 4 inhibitors could be useful in the treatment of various diseases. To identify new and more potent inhibitors, 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 μ M) that are capable of inhibiting the activity of the protein as low as Arachidonic acid (FABP4i IC_{50} 3.42 ± 0.54 μ M). 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.

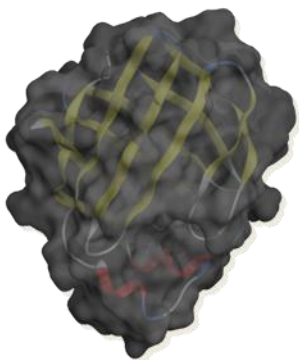
WHY DO WE WANT TO INHIBIT THIS PROTEIN?

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. II-FABP | (ileal) |
| 7. B-FABP | (brain) |
| 8. M-FABP | (myelin) |
| 9. T-FABP | (testis) |

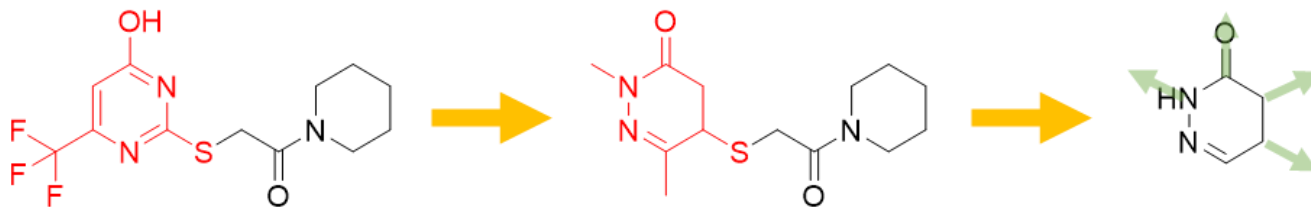
Modified FABPs expression patterns were described for prostate, bladder, renal cell carcinoma and other types of cancer cells:

- **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

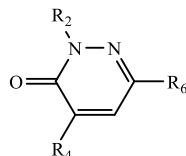
To generate a novel series of FABP4 inhibitors we have exploited a two-step computing assisted molecular design. As showed below, in the first step of the drug-design process we focused on the search for bioisosteric replacements/scaffold-hopping of the pyrimidine scaffold of the co-crystallized ligand (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



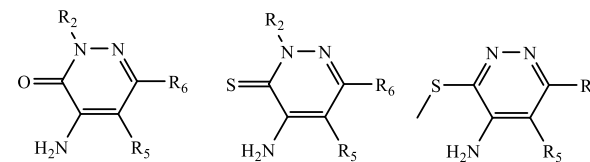
Results and discussion

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 .

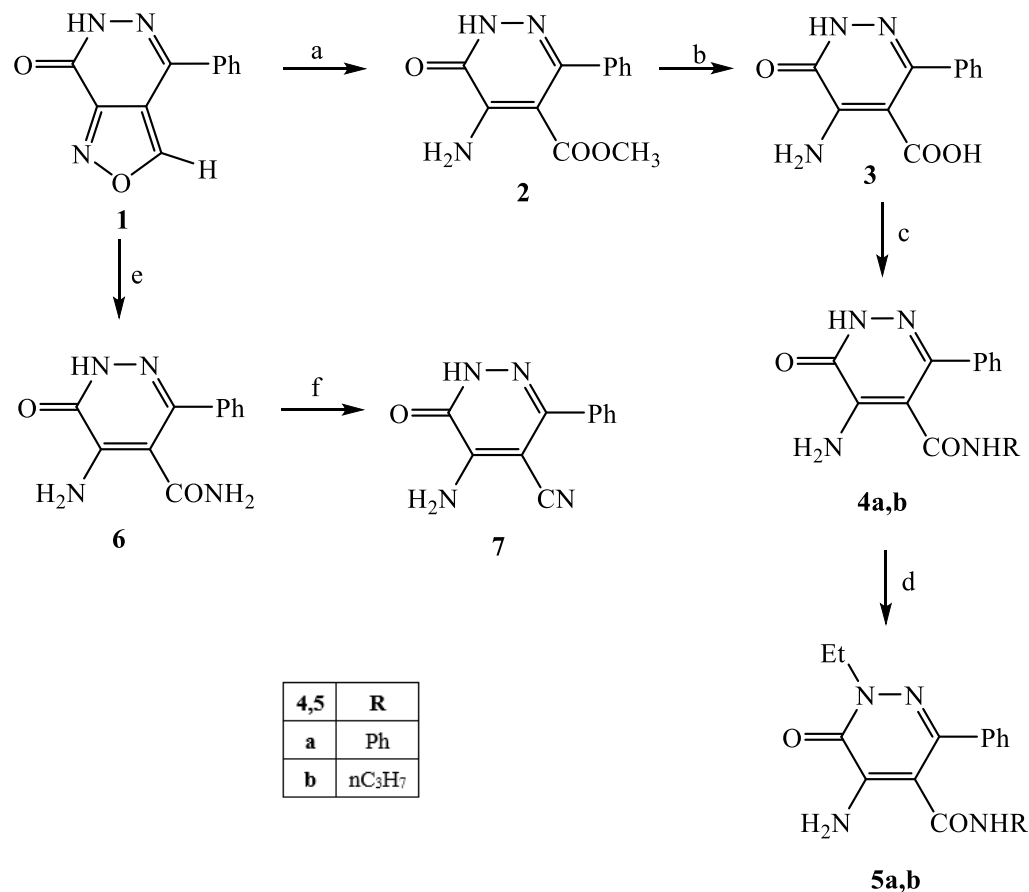
Results and discussion



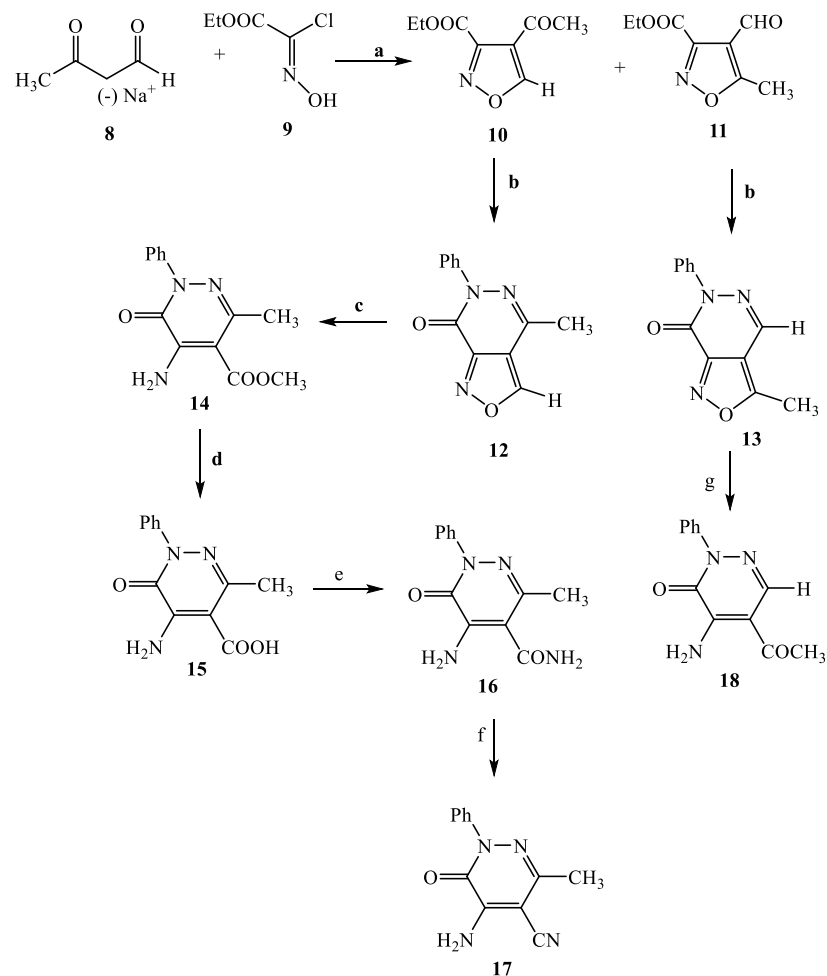
| Comp. | R ₂ | R ₄ | R ₆ |
|-------|---------------------------------|------------------------------------|---------------------------------|
| 25a | CH ₃ | NHCONH ₂ | Ph |
| 25b | cC ₆ H ₁₁ | NHCONH ₂ | Ph |
| 25c | C ₂ H ₅ | NHCONH ₂ | Ph |
| 25d | iC ₃ H ₇ | NHCONH ₂ | Ph |
| 25e | nC ₃ H ₇ | NHCONH ₂ | Ph |
| 25f | nC ₄ H ₉ | NHCONH ₂ | Ph |
| 28 | H | NHCONH ₂ | Ph |
| 29a | CH ₃ | NHCOCH ₃ | Ph |
| 29b | CH ₃ | NHCOC ₂ H ₅ | Ph |
| 29c | CH ₃ | NHCOiC ₃ H ₇ | Ph |
| 29d | CH ₃ | NHCONC ₃ H 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 ₆ H ₁₁ |
| 39b | CH ₃ | NHCONH ₂ | iC ₃ H 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 |



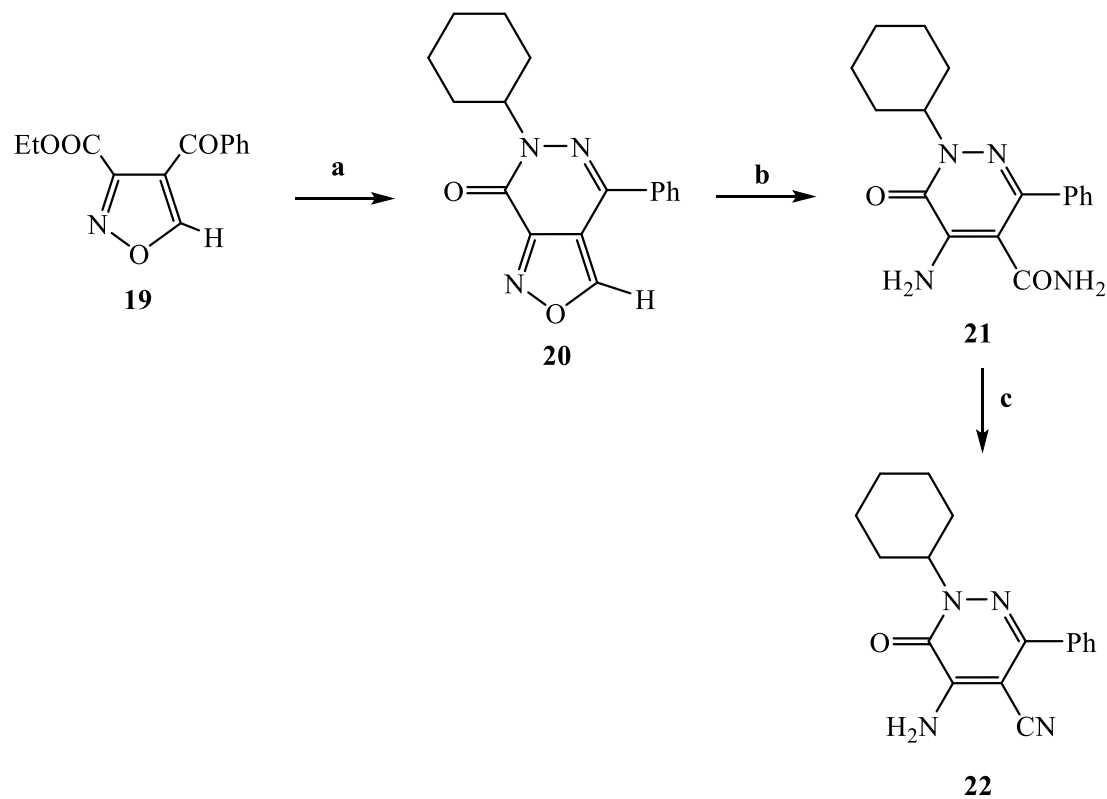
| Comp. | R ₂ | R ₅ | R ₆ |
|-------|---------------------------------|------------------------------------|---------------------------------|
| 4a | H | CONHPh | Ph |
| 4b | H | CONHnC ₃ H ₇ | Ph |
| 5a | C ₂ H ₅ | CONHPh | Ph |
| 5b | C ₂ H ₅ | CONHnC ₃ H ₇ | Ph |
| 6 | H | CONH ₂ | Ph |
| 7 | H | CN | Ph |
| 16 | Ph | CONH ₂ | CH ₃ |
| 17 | Ph | CN | CH ₃ |
| 18 | Ph | COCH ₃ | H |
| 21 | cC ₆ H ₁₁ | CONH ₂ | Ph |
| 22 | cC ₆ H ₁₁ | CN | Ph |
| 24d | iC ₃ H ₇ | H | Ph |
| 24e | nC ₃ H ₇ | H | Ph |
| 24f | nC ₄ H ₉ | H | Ph |
| 27 | - | H | Ph |
| 32 | CH ₃ | H | Ph |
| 37a | H | H | 3-thienyl |
| 37c | H | H | cC ₆ H ₁₁ |
| 37d | H | H | iC ₃ H ₇ |
| 38a | CH ₃ | H | 3-thienyl |
| 38b | CH ₃ | H | cC ₆ H ₁₁ |
| 38c | CH ₃ | H | iC ₃ H 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 |



Reagents and conditions: (a) Et₃N, CH₃OH, 60 °C, 2 h; (b) 6N NaOH, EtOH, reflux, 30 min; (c) (i) SOCl₂, Et₃N, r.t., 30 min; (ii) R-NH₂, anhydrous THF, r.t., 2 h; (d) CH₃CH₂Br, K₂CO₃, anhydrous DMF, reflux, 30-90 min; (e) 33% NH₄OH, C₅H₁₁N, 60 °C, 90 min; (f) POCl₃, 60 °C, 2h.

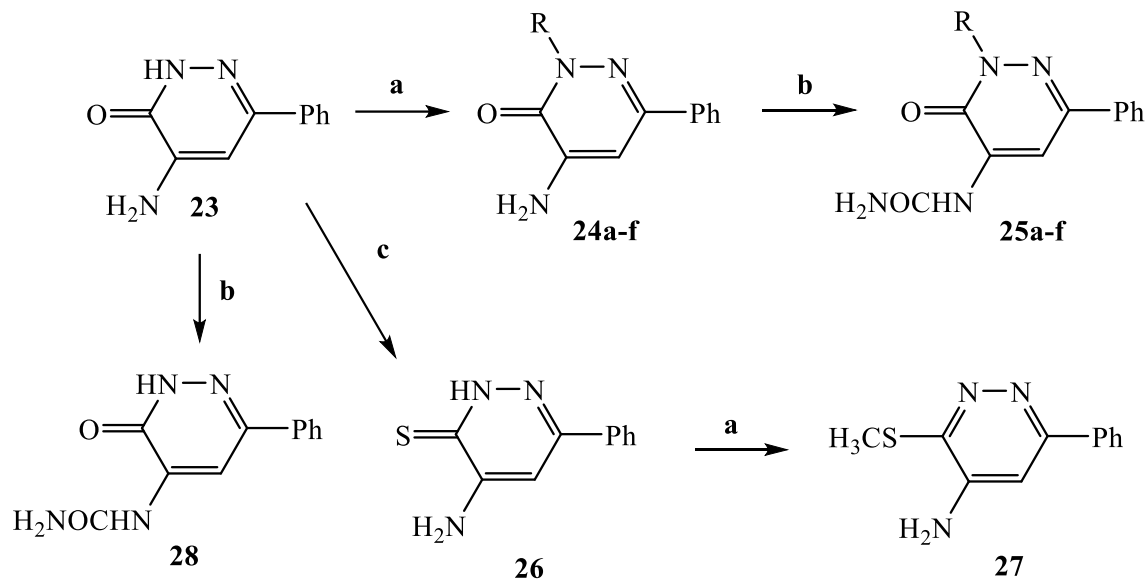


***Reagents and conditions:** (a) anhydrous EtOH, 0 °C, 1h; (b) phenylhydrazine, PPA, EtOH, 70 °C, 30 min; (c) CH₃OH, Et₃N, 60 °C, 2h; (d) NaOH, EtOH, reflux, 30 min; (e) (i) SOCl₂, Et₃N, reflux, 30 min.; (ii) 33% NH₄OH, anhydrous THF, r.t., 15 min; (f) POCl₃, 60 °C, 2h; (g) HCOONH₄, 10% Pd/C, EtOH, reflux, 2 h.

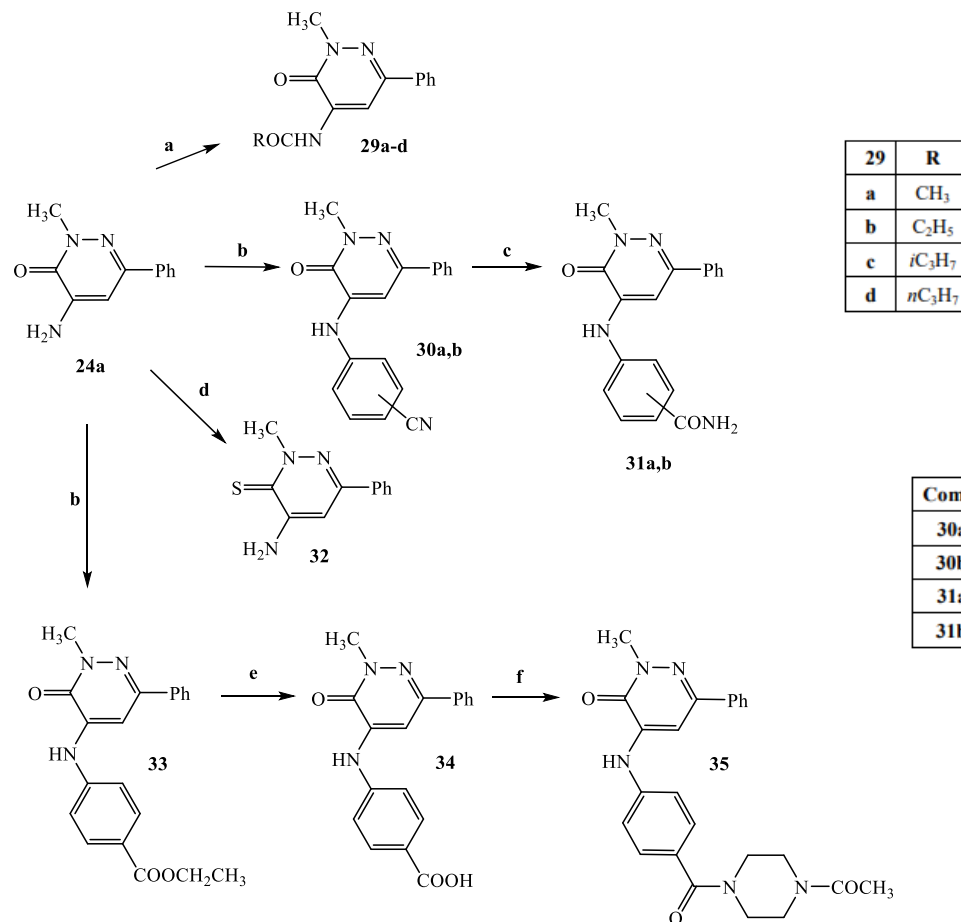


^a**Reagents and conditions:** (a) cyclohexylhydrazine, PPA, EtOH, 70 °C, 30 min; (b) 33% NH₃, piperidine, 60 °C, 90 min; (c) POCl₃, 60 °C, 2h.

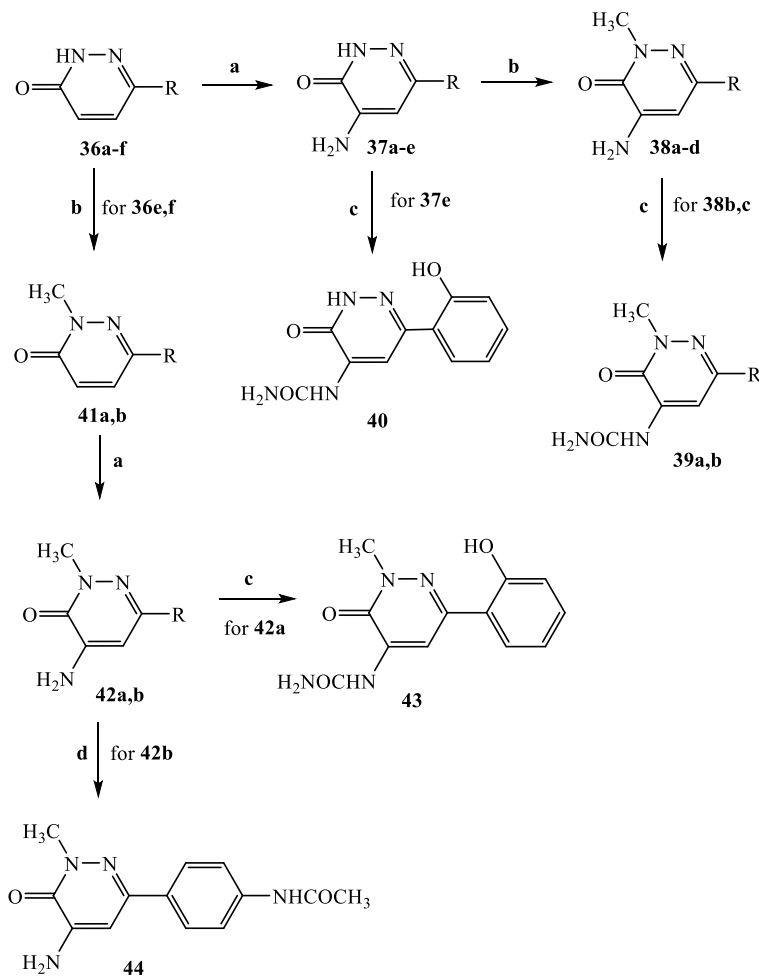
| 24,25 | R |
|-------|---|
| a | CH ₃ |
| b | <i>c</i> C ₆ H ₁₁ |
| c | C ₂ H ₅ |
| d | <i>i</i> C ₃ H ₇ |
| e | <i>n</i> C ₃ H ₇ |
| f | C ₄ H ₉ |



^aReagents and conditions: (a) suitable R-Br, K₂CO₃, anhydrous DMF, reflux, 1-4 h; (b) (i) dry THF, CH₃COONa, 0 °C then triphosgene, reflux, 2 h; (ii) NH₃ 33%, 0 °C, 1 h; (c) Lawesson's reagent, anhydrous toluene, reflux, 5 h.



Reagents and conditions: (a) suitable (R-CO₂)O, anhydrous C₆H₅N, closed tube, 140 °C, 5 h; (b) 2/3-cyanophenylboronic acid (for 30a,b) or 4-ethoxycarbonylphenylboronic acid (for 33), Cu(Ac)₂, Et₃N, dry CH₂Cl₂, r.t., 12 h; (c) H₂SO₄ 80%, 80 °C, 4 h; (d) Lawesson's reagent, anhydrous toluene, reflux, 10 h; (e) NaOH 6N, EtOH 96%, reflux, 1 h; (f) (i) SOCl₂, Et₃N (catalytic), reflux, 1 h; (ii) anhydrous THF, 1-acetypiperazine, 0 °C then r.t., 1 h.



| 39 | R |
|----|----------------------------|
| a | $c\text{C}_6\text{H}_{11}$ |
| b | $i\text{C}_3\text{H}_7$ |

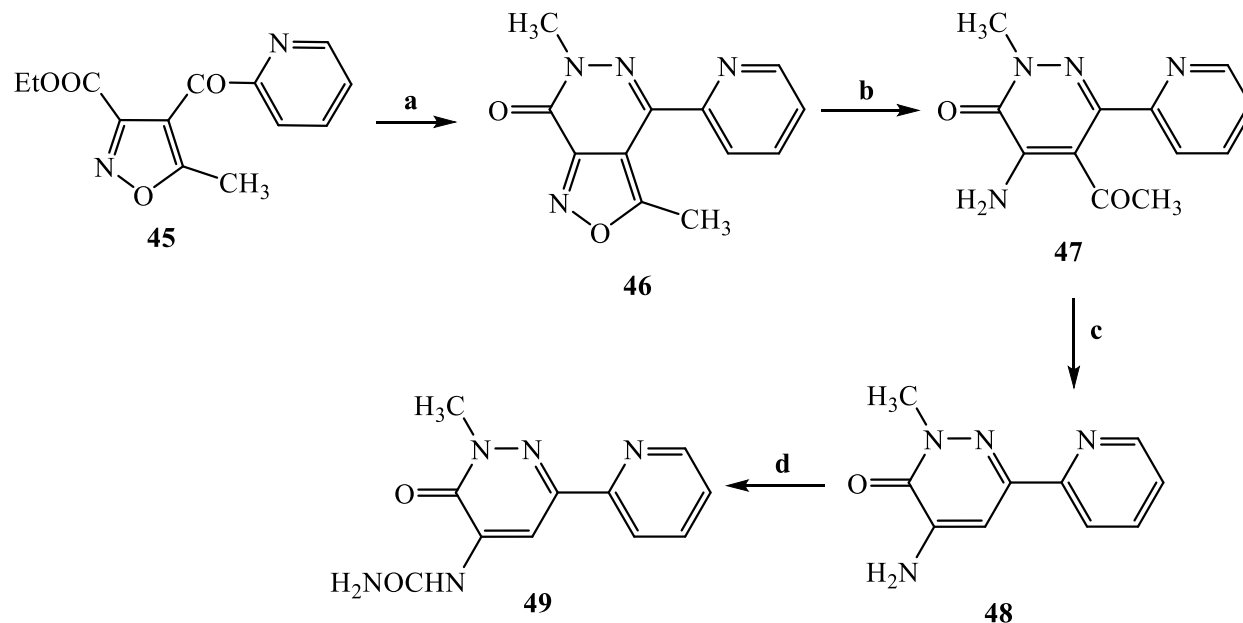
| 36 | R |
|----|----------------------------|
| a | 3-thienyl |
| b | $c\text{C}_6\text{H}_{11}$ |
| c | $i\text{C}_3\text{H}_7$ |
| d | CH_2Ph |
| e | 2-OH-Ph |
| f | 4-NO ₂ -Ph |

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

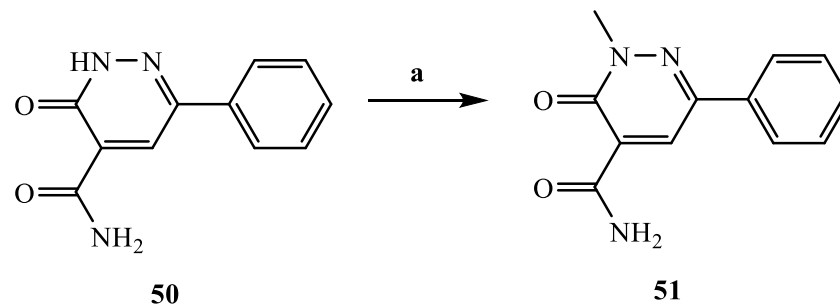
| 37 | R |
|----|----------------------------|
| a | 3-thienyl |
| b | $c\text{C}_6\text{H}_{11}$ |
| c | $i\text{C}_3\text{H}_7$ |
| d | CH_2Ph |
| e | 2-OH-Ph |

| 38 | R |
|----|----------------------------|
| a | 3-thienyl |
| b | $c\text{C}_6\text{H}_{11}$ |
| c | $i\text{C}_3\text{H}_7$ |
| d | CH_2Ph |

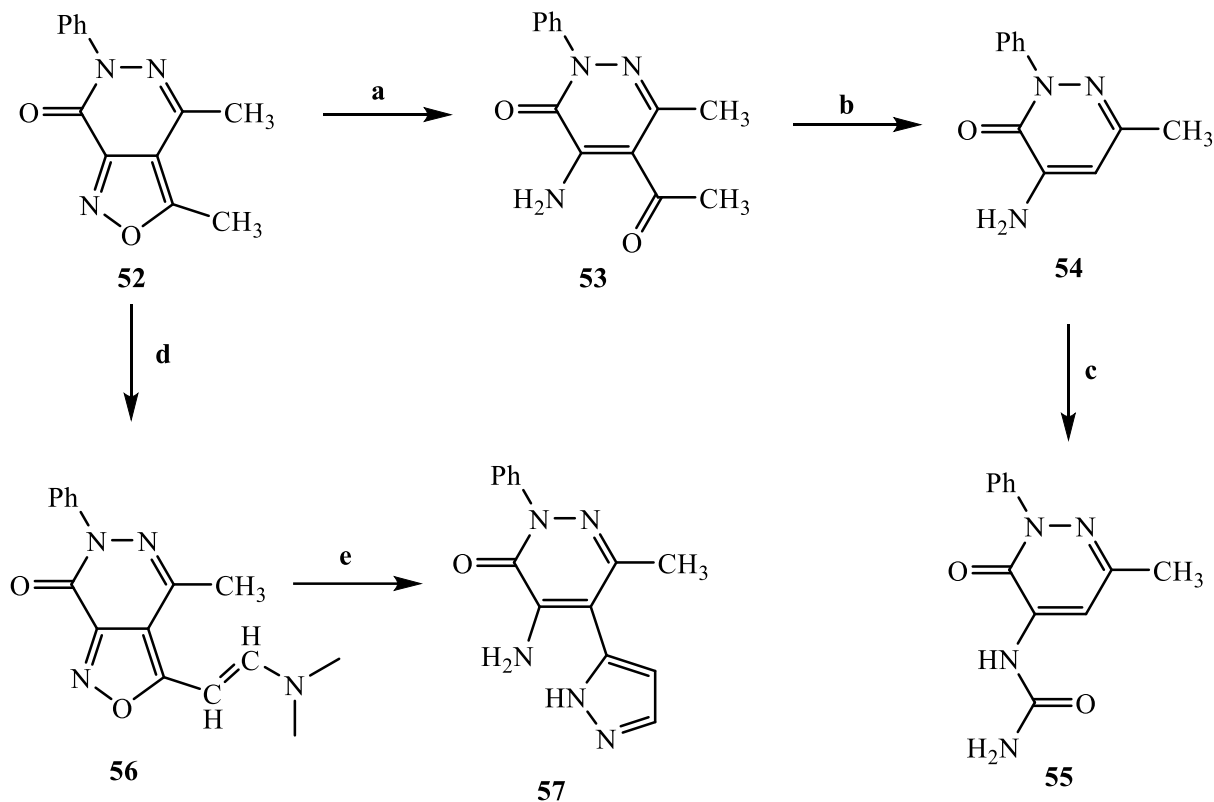
***Reagents and conditions:** (a) $\text{NH}_2\text{NH}_4 \cdot \text{H}_2\text{O}$, sealed/pressure vessel, 180 °C, 12 h; (b) CH_3I , K_2CO_3 , anhydrous DMF, 80 °C, 2-4 h; (c) (i) anhydrous THF, CH_3COONa , 0 °C then triphosgene, reflux, 2 h; (ii) NH_4OH 33%, 0 °C, 1 h; (d) ClCOCH_3 , anhydrous THF, 0 °C, then r.t., 20 min



^aReagents and conditions: (a) $\text{CH}_3(\text{NH})\text{NH}_2$, 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.

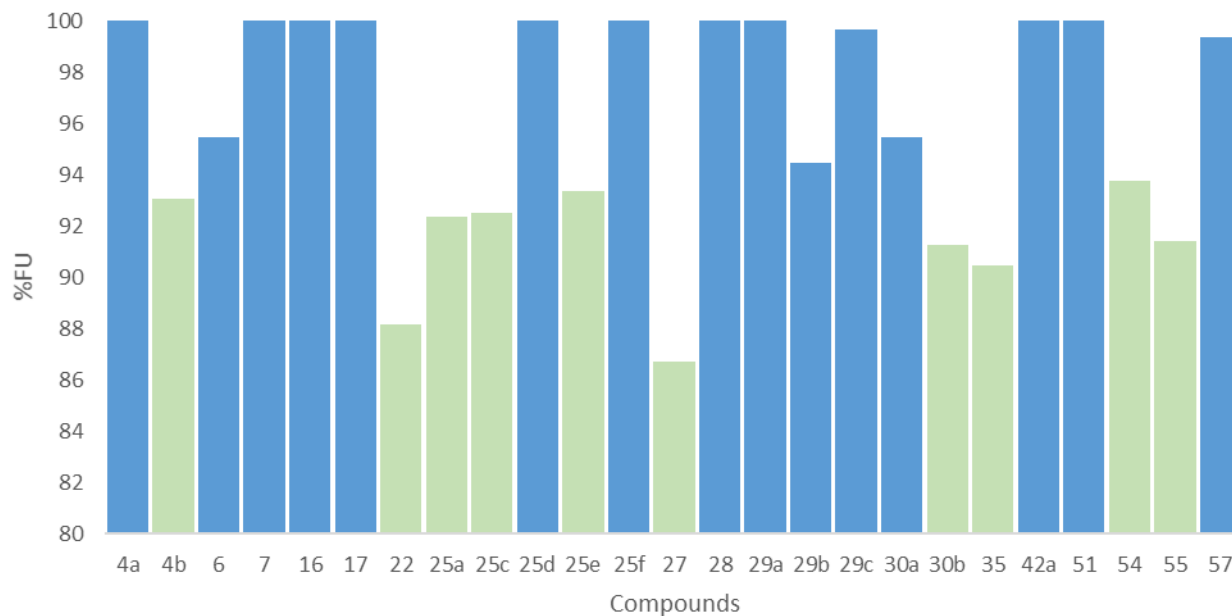


^aReagents and conditions: (a) CH₃I, K₂CO₃, anhydrous DMF, 80 °C, 2 h.



^aReagents 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) $\text{NH}_2\text{NH}_4 \cdot \text{H}_2\text{O}$, anhydrous EtOH, 70 °C, 10 h.

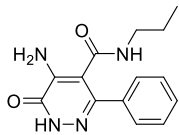
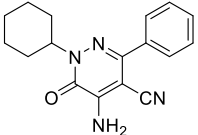
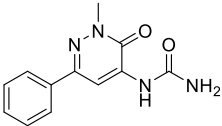
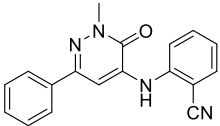
Results and discussion



Single point displacement experiment for selected compounds

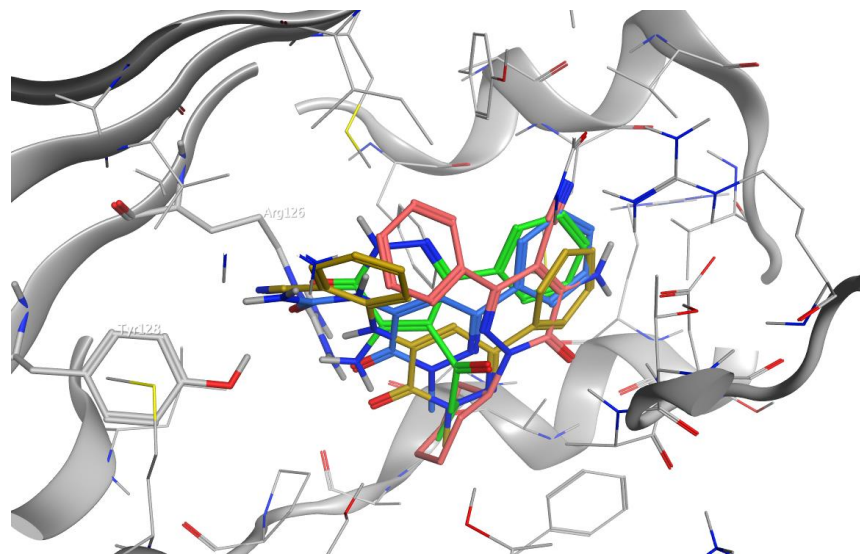
Results and discussion

Measured IC₅₀ values for selected compounds

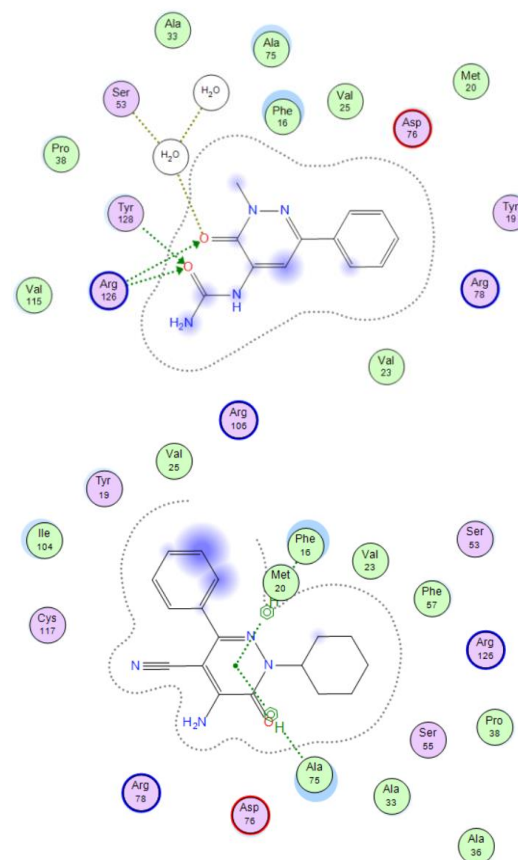
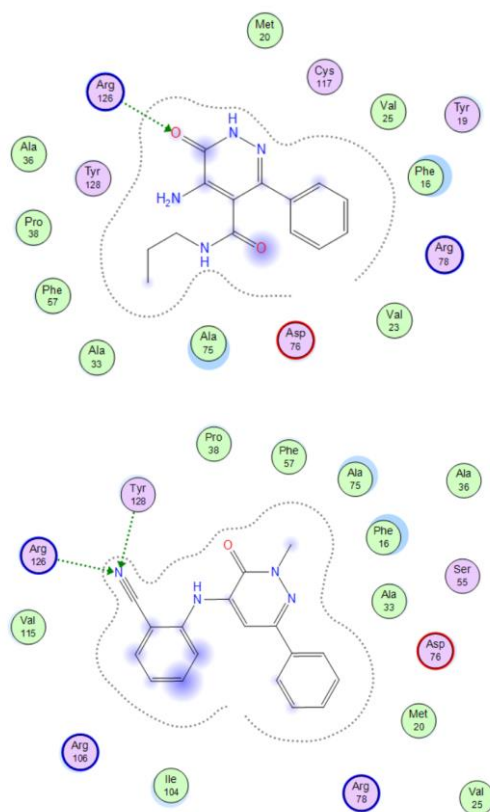
| Compounds | | IC ₅₀ (μM) |
|---|------------|-----------------------|
|  | 4b | 3.42 ± 0.54 |
|  | 22 | 8.27 ± 0.20 |
|  | 25a | 2.97 ± 0.26 |
|  | 30b | 23.18 ± 0.52 |
| | 22 | 15.23 ± 0.76 |
| | 25c | >50 |
| | 35 | >50 |
| | 25e | >50 |
| | 54 | >50 |
| | 55 | >50 |
| | 27 | >50 |

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 μ M, which is higher than the positive control, docking experiments confirmed the interaction with the binding pocket of the protein