Bioorganic & Medicinal Chemistry 20 (2012) 3429–3445

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Pyrazole[3,4-*e*][1,4]thiazepin-7-one derivatives as a novel class of Farnesoid X Receptor (FXR) agonists

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ARTICLE INFO

Article history:
Received 13 February 2012
Revised 2 April 2012
Accepted 7 April 2012
Available online 20 April 2012

Keywords:
Farnesoid X Receptor (FXR)
Non-steroidal FXR agonist
Virtual screening
1*H*-Pyrazole[3,4-*e*][1,4]thiazepin-7-one
Heterocyclic chemistry

ABSTRACT

A virtual screening procedure was applied to the discovery of structurally diverse non-steroidal Farnesoid X Receptor (FXR) agonists. From 117 compounds selected by virtual screening, a total of 47 compounds were found to be FXR agonists, with 34 of them showing activity below a concentration of 20 μM. 1*H*-Pyrazole[3,4-*e*][1,4]thiazepin-7-one-based hit compound **7** was chosen for hit-to-lead optimization. A large number of 1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one derivatives was designed, synthesized, and evaluated by a cell-based luciferase transactivation assay for their agonistic activity against FXR. Most of them exhibited low micromolar range of potency and very high efficacy.

1. Introduction

Farnesoid X Receptor (FXR) belongs to a family of nuclear receptors that are ligand-inducible transcription factors. FXR is highly expressed in the liver and intestine and is activated by bile acids, such as chenodeoxycholic acid (CDCA) and cholic acid (CA), and their corresponding conjugates, at physiological concentrations.[1–3] Activated FXR binds with high affinity to an inverted repeat-1 (IR-1) response element on DNA as a heterodimer with the retinoid X receptor (RXR) regulating in such a way the expression of various transport proteins and biosynthetic enzymes that maintain cholesterol and bile acid homeostasis.[4] Indeed, activation of FXR by bile acids or synthetic agonists results in (a) transcriptional repression of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the bile acid biosynthesis pathway, (b) induction of the small heterodimer partner (SHP), a transcriptional repressor found in the liver and intestine, and (c) induction of genes encoding for some bile acid transport proteins, such as intestinal bile acid-binding protein (IBABP)[5] and bile salt export pump (BSEP).[6] Furthermore, bile acids-mediated FXR activation has been recently recognized as a major underlying pathway for energy homeostasis and glucose and lipids metabolism.[7] FXR has also been suggested to counteract pro-inflammatory and pro-atherogenic responses in cardiovascular diseases.[8] Moreover, the bile acid-FXR interaction plays a pivotal role in regulating liver inflammation and regeneration as well as in regulating extent of inflammatory responses, barrier function and prevention of bacterial translocation in the intestinal tract.

All these evidences make FXR a promising potential target for the treatment of a variety of metabolic disorders, including hyperlipidemia, cholelithiasis, cholestasis, and diabetes mellitus.[9]

Over the past few years, many efforts have been dedicated to the search of highly potent steroidal- and non-steroidal FXR modulators (Fig. 1). Based on structural modification of CDCA, our group reported in 2002, 6α-ethylchenodeoxycholic acid (obeticholic acid, INT-747, **1**) as a highly potent and orally available FXR full agonist.[10] Positive data from two phase II clinical trials using INT-747 (**1**), recently demonstrated the clinical utility of this compound in the treatment of primary biliary cirrhosis, a chronic inflammatory cholestatic condition in the liver, and type 2 diabetes.

In addition to steroidal FXR agonist, such as INT-747 (**1**) and MFA-1 (**2**),[11] a number of synthetic non-steroidal FXR agonists have been reported in the literature in last decade.

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http://dx.doi.org/10.1016/j.bmc.2012.04.021

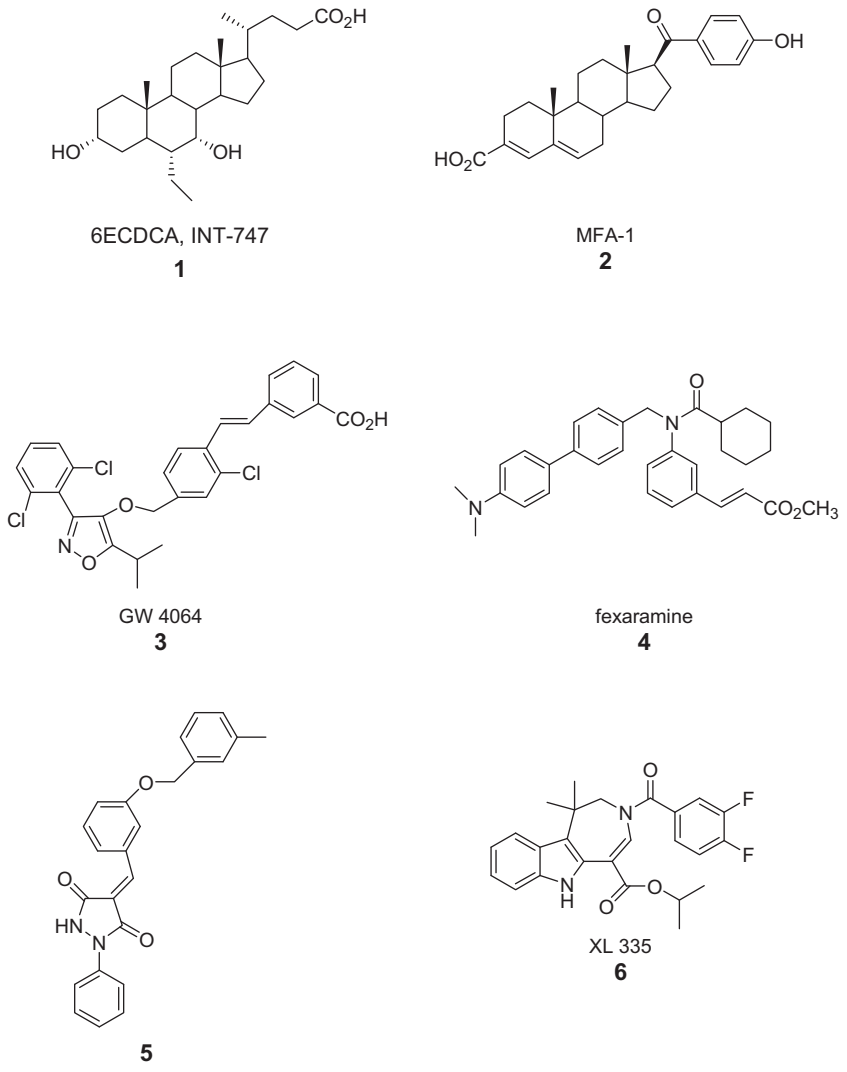


Figure 1. Structures of selected steroidal- and non-steroidal FXR agonists.

GW4064 (**3**) was the first high affinity non-steroidal FXR agonist to be reported in 2000 by Maloney et al.[12] Although this compound suffered from the presence in its structure of a potential toxicophore such as the *trans*-stilbene moiety, that is also responsible for UV light instability of the compound, and from a poor oral bioavailability, **3** was the first chemical tool to demonstrate potent FXR binding and activation in biochemical and cellular in vitro assays as well as pharmacological effects in different rodent models of metabolic diseases. Also the optimization of initial hits identified by the screening of natural product-like libraries provided a number of potent FXR agonists, fexaramine (**4**), in particular.[13] Another recent example is the identification of bioactive triterpenes from *Ganoderma lucidum*.[14,15]

Since these first reports a large amount of work has been focused towards the identification of novel non-steroidal FXR agonists whether by the modification of the prototype GW4064 (**3**) or by the exploration of structurally different scaffolds. Following the first approach, the structural modifications were focused on the replacement of the stilbene side-chain of the prototype,[16] as well as around the 3-position of the isooxazole ring, that represents the heterocyclic core of the compound.[17] Whereas the search for novel structurally diverse FXR agonists resulted in the identification of a novel pyrazoline-3,5-dione-based scaffold hit compound **5**[18] and in a novel series of azepino[4,5-*b*]indoles leading up to the clinical candidate XL335 (WAY-362450, FXR-450, **6**).[19]

Starting from a virtual screening study that successfully has led to identify novel non-steroidal classes of FXR modulators, herein we report the synthetic efforts aimed to optimize the activity of the pyrazole[3,4-*e*][1,4]thiazepin-7-one-based class of compounds. While instrumental to depict structure–activity relationships within this class of FXR ligands, the results have allowed to disclose ***s*(±)24**, ***s*(±)37** and ***s*(±)38** as novel FXR agonists in low micromolar range of potency and with very high efficacy.

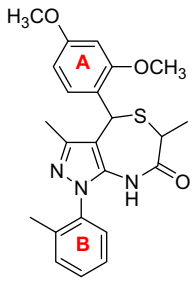
2. Results and discussion

2.1. Virtual screening

A virtual screening experiment has been carried out using the crystal structures of FXR in complex with agonists. In particular, a two-step approach was adopted for this part of the study, using firstly a ligand-based filtering and then a structure-based screening. The ligand-based filtering was carried out by developing a pharmacophoric model of the ligand-target interactions observed in five selected X-ray structures of FXR (pdb codes: 3BEJ,[11] 1OSH,[13] 1OSV,[10] 3DCT,[16] and 3DCU[16]) as detailed in the method

section. The particularity of our approach, with respect to others already published,[14,15] resides in the contemporary usage of all the ligands resumed in a 'supermolecule' coming from X-ray complexes and use it as starting point for the features' mapping. As a result, the pharmacophore model was composed of 13 interaction points, including 2 hydrogen bond acceptors, 1 hydrogen bond donor, 7 aromatic points, 1 hydrophobic centre, 2 negative charged points (Fig. 2A). Excluded volumes were also added using the residues of the aligned binding site as detailed in the method section (Fig. 2B).

Then, three million of commercially available compounds from the DrugLike (2 million) and the LeadLike (1 million) subsets of the ZINC 8 database[20] were funnelled through the pharmacophoric model. We decided to use the ZINC database, with respect to other data sources, to manage a single comprehensive repository covering nearly 45 compound vendors, furthermore already divided in 'ready to use' subsets with LeadLike and DrugLike properties. Thus, 760 compounds (630 from the Druglike dataset and 130 from the Leadlike dataset) were identified as virtual hits, matching a minimum number of 7 interaction sites out of 13. These compounds were further submitted to a structure-based screening, consisting in a cross-docking of the molecules into three FXR structures with two different programs, namely GLIDE 5.5[21] in extra precision docking mode (XP) and Leadfinder[22] in docking mode, as detailed in the method section (pdb codes: 3BEJ,[11] 1OSV,[10] 3DCT[16]). The aim of using different programs and crystal structures was firstly to overcome the limits of the single programs in the parametrization of the interactions and secondly, to better cover the receptor flexibility on the side-chains of the binding site. From the resulting list of docking scores, we selected those compounds endowed with scoring values better than those of the co-crystallized FXR ligands used for the pharmacophore generation. Accordingly, the selected virtual hits were 263 from GLIDE docking and 256 from Leadfinder docking, with 178 the compounds being present in both scoring lists. The removal of the ligands with more than two chiral centres and duplicates led to a final set of 328 virtual hits. To select the most chemically diverse compounds, a clustering analysis with a Tanimoto similarity factor[23] of 0.8 was performed on the resulting hits, and 149 representative compounds were selected for purchasing. Among them, 117 molecules were successfully retrieved and tested by AlphaScreen assay.[24] As a result, 47 active hit compounds were identified, with 34 of them showing activity below a concentration of 20 μM. The analysis of these compounds involved several criteria, including chemical novelty, synthetic versatility, solubility and chemical stability. After such analysis, 3,6-dimethyl-1-(2-methylphenyl)-4-(2,4-dimethoxyphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one (**7**, EC_{50} = 10.5 μM, efficacy = 63%) was selected as a new scaffold for a further hit-to-lead optimization on the way to novel FXR modulators.



7

2.2. Hit optimization

Starting from the hit **7**, we planned a chemical strategy with the aim of exploring substitution patterns of rings A and B. Although the main goal was to optimize both potency and efficacy of **7**, a second objective was the development of a structure–activity relationship (SAR) scheme for this class of compounds and use this information to infer a putative binding mode of **7** into the receptor. Accordingly, a first series of dihydro-1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-one derivatives were synthesized and evaluated for their agonistic activity at FXR. As shown in Table 1, in this series we explored the effects of modifications on the aromatic ring A of **7** by the synthesis of derivatives **8**–**34** (series I).

In agreement with virtual screening results, the *anti*-couples proved to be less active than the corresponding *syn*-ones demonstrating that the relative disposition of the substituents is a crucial parameter for the activity. Accordingly, we will only comment on the biological results of the *syn*-couples. As shown in Table 1, many of the compounds belonging to the first series (15 out of 24) showed agonistic activity higher than that of the hit **7**. In particular, the initial replacement of the 2,4-dimethoxy substituent with 4-biphenyl group was found to increase the FXR potency ~10-fold, although a concomitant reduction in efficacy was observed [cf. ***s*(±)8** vs **7**].

The introduction of substituents on the distal phenyl group of ***s*(±)8** afforded different results depending from position and substituent properties. *meta*- and *para*-Substitutions by carboxylic acid and ester [***s*(±)13–16**]) as well as by styryl moiety caused a completely loss of activity. The presence of a methoxy group in *ortho*- [***s*(±)9**], *meta*- [***s*(±)10**] and *para*-positions [***s*(±)11**] was not influential for the activity with respect to ***s*(±)8**. The replacement of the distal phenyl group of ***s*(±)8** by heterocycles, such as 2- and 3-thiophenes [***s*(±)18** and ***s*(±)19**, respectively], 2- and 3-furanes [***s*(±)20** and ***s*(±)21**, respectively] and 2-benzofurane [***s*(±)22**] was more or less neutral in terms of EC_{50}, whereas resulted in a

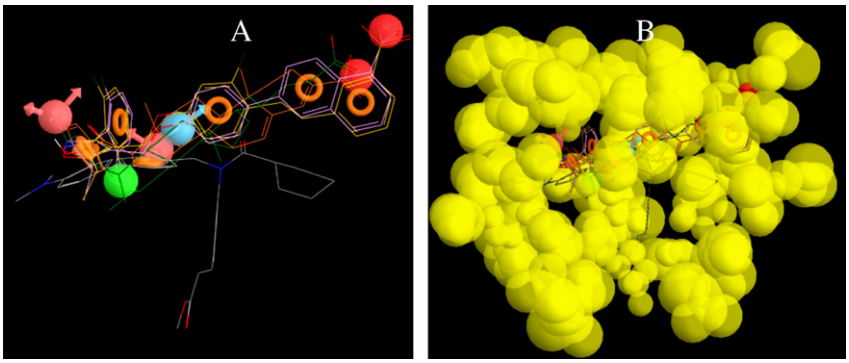
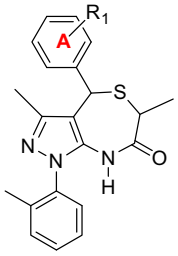


Figure 2. (A) Pharmacophore hypothesis: 2 hydrogen bond acceptors (red dot with arrows), 1 hydrogen bond donor (cyan dot with arrow), 7 aromatic points (orange circle), 1 hydrophobic centre (green dot), 2 negative charges (red dot). (B) Excluded volumes (yellow dots) generated by the superimposed backbones of FXR.

Table 1
FXR functional activity for dihydro-1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-ones of series I, **8–34**



8-34

Compound[a]	R_1	EC_{50}[b] (μM)	Efficacy[c] (%)
7	2,4-Dimethoxy	10.5 ± 0.5	63 ± 9
***s*(±)8**	4-Phenyl	0.99 ± 0.1	24 ± 7
***s*(±)9**	4-(2-Methoxyphenyl)	1.1 ± 0.3	50 ± 3
***s*(±)10**	4-(3-Methoxyphenyl)	1.8 ± 0.1	41 ± 7
***s*(±)11**	4-(4-Methoxyphenyl)	1.2 ± 0.2	38 ± 10
***s*(±)12**	4-(3-Ethoxyphenyl)	3.5 ± 0.4	13 ± 2
***s*(±)13**	4-(3-Carbomethoxyphenyl)	NA[d]	
***s*(±)14**	4-(4-Carbomethoxyphenyl)	NA[d]	
***s*(±)15**	4-(3-Carboxyphenyl)	NA[d]	
***s*(±)16**	4-(4-Carboxyphenyl)	NA[d]	
***s*(±)17**	4-(*E*-Styryl)	NA	
***s*(±)18**	4-(2-Thienyl)	2.6 ± 0.4	85 ± 3
***s*(±)19**	4-(3-Thienyl)	1.4 ± 0.5	68 ± 4
***s*(±)20**	4-(2-Furanyl)	3.6 ± 0.2	32 ± 4
***s*(±)21**	4-(3-Furanyl)	3.5 ± 0.5	40 ± 2
***s*(±)22**	4-(2-Benzofuranyl)	1.5 ± 0.5	17 ± 4
***s*(±)23**	4-Phenoxy	1.3 ± 0.5	118 ± 4
***s*(±)24**	4-(2-Methoxyphenoxy)	1.7 ± 0.4	240 ± 20
***s*(±)25**	4-(3-Methoxyphenoxy)	1.7 ± 0.1	120 ± 2
***s*(±)26**	4-(4-Methoxyphenoxy)	2.2 ± 0.1	70 ± 20
***s*(±)27**	4-(2-Carbomethoxyphenoxy)	NT[e]	
***s*(±)28**	4-(3-Carbomethoxyphenoxy)	NT[e]	
***s*(±)29**	4-(4-Carbomethoxyphenoxy)	NT[e]	
***s*(±)30**	4-(2-Carboxyphenoxy)	NA[d]	
***s*(±)31**	4-(3-Carboxyphenoxy)	NA[d]	
***s*(±)32**	4-(4-Carboxyphenoxy)	NA[d]	
***s*(±)33**	4-Benzyloxy	NA[d]	
***s*(±)34**	3-Methoxy-4-phenoxy	1.9 ± 0.1	130 ± 7

[a] The symbol *s* refers to the racemic couples endowed with *syn*-disposition of the substituents at C-4 and C-6 positions. The *anti*-couples resulted in any case less active than the corresponding *syn*-couples. Data relative to the *anti*-couples are not shown.
[b] Ligand-dependent recruitment of Src-1 peptide assessed by AlphaScreen assay. hFXR-LBD-GST was incubated with increasing concentrations of the indicated ligand in the presence of biotinylated Src-1 peptide. The AlphaScreen signal increases when the complex receptor-co-activator is formed.[24]
[c] Efficacy: % of compound effect versus 10 μM of CDCA.
[d] NA = not active.
[e] NT = not tested because available in poor amount.

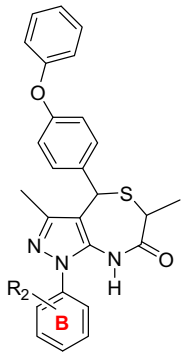
substantial increase in efficacy in the case of 2-thienyl derivative ***s*(±)18**. An encouraging result was obtained by the insertion of an oxygen bridge between the two ring of the biphenyl moiety of ***s*(±)8**: the resulting derivative ***s*(±)23** indeed, showed to be a full agonist with a potency ~10-fold when compared to the hit **7** and an efficacy of 118% versus 10 μM of CDCA. By maintaining the positive ether linkage, different substitutions on the distal phenyl ring were then explored; notably, the introduction of a methoxy group at *ortho*-position resulted in about twofold increase in efficacy [cf. ***s*(±)24** vs ***s*(±)23**]. The *meta*-substituted methoxy derivative ***s*(±)25** was equipotent with ***s*(±)23**, whereas a slight decrease in efficacy was observed with the corresponding *para*-substituted derivative [cf. ***s*(±)26** vs ***s*(±)23**]. The decoration of the proximal aromatic ring of ***s*(±)23**, exemplified by the introduction of a methoxy group in *meta*-position, was neutral in potency as well as in efficacy [cf. ***s*(±)31** vs ***s*(±)23**]. The introduction of one-carbon spacer between the distal phenyl group and the oxygen linkage of ***s*(±)23** resulted in the inactive derivative ***s*(±)30**.

By choosing the 4-(phenoxy)phenyl moiety as the reference substitution at C4-position of the pyrazolo[3,4-*e*][1,4]thiazepine-7-one core, we moved to explore different substitutions on phenyl ring B by the synthesis of compounds **35–45** belonging to the series II (Table 2).

Firstly, we shifted the methyl group of ***s*(±)23** from the *orto*- to the *meta*- and *para*-positions of ring B [***s*(±)35** and ***s*(±)36**]. As a result, both *meta*- and *para*-methyl analogues of ***s*(±)23** showed a drop in efficacy, while keeping the EC_{50} in the same range of potency of the parent compound.

Accordingly, a number of substituents endowed with different electronic and steric properties were introduced at the *ortho*-position of ring B. The biological appraisals of these derivatives showed that alkyl groups (Et, *n*Pr and *i*Pr) contributed positively to the efficacy [cf. ***s*(±)37–39** vs ***s*(±)23**], with the *n*-propyl analogue being the most active compound **[(*s*(±)38]**. Among halogenated derivatives (**41–44**), the iodine-substituted derivative (**44**), showing an efficacy of 130%, appeared particularly promising in consideration that it was tested as a mixture of four diastereoisomers. Conversely, the introduction of methoxy- or trifluoromethyl groups afforded compounds [cf. ***s*(±)45** and **40** vs ***s*(±)23**] with poor efficacy.

Table 2
FXR functional activity for dihydro-1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-ones of series II, **35–45**



35-45

Compound	R_2	EC_{50}[c] (μM)	Efficacy[d] (%)[a]
***s*(±)23**[a]	2-CH_3	1.3 ± 0.5	118 ± 4
***s*(±)35**[a]	3-CH_3	2.4 ± 0.5	22 ± 2
***s*(±)36**[a]	4-CH_3	3.4 ± 1.4	11 ± 2
***s*(±)37**[a]	2-CH_2CH_3	1.1 ± 0.1	190 ± 10
***s*(±)38**[a]	2-$CH_2CH_2CH_3$	1.1 ± 0.3	240 ± 50
***s*(±)39**[a]	2-$CH(CH_3)_2$	1.9 ± 0.1	174 ± 20
40[b]	2-CF_3	2.0 ± 0.3	50 ± 3
41[b]	2-F	3.9 ± 0.1	72 ± 10
42[b]	2-Cl	2.7 ± 0.2	57 ± 5
43[b]	2-Br	1.8 ± 0.2	84 ± 6
44[b]	2-I	1.3 ± 0.3	130 ± 20
***s*(±)45**	2-OCH_3	3.3 ± 0.9	37 ± 4

[a] The symbol *s* refers to the racemic couple endowed with *syn*-disposition of the substituents at C-4 and C-6 positions. The *anti*-couples resulted in any case less active than the corresponding *syn*-couples. Data relative to the *anti*-couples are not shown.
[b] Compound tested as mixture of *syn*- and *anti*-couples.
[c] Ligand-dependent recruitment of Src-1 peptide assessed by AlphaScreen assay. hFXR-LBD-GST was incubated with increasing concentrations of the indicated ligand in the presence of biotinylated Src-1 peptide. The AlphaScreen signal increases when the complex receptor-coactivator is formed.[24]
[d] Efficacy: % of compound effect versus 10 μM of CDCA.

2.3. Gene expression profile

In view of their efficacy values, compounds ***s*(±)24**, ***s*(±)37** and ***s*(±)38** were selected to evaluate their capacity to modulate selected FXR target genes with respect to obeticholic acid (INT-747, **1**) (Fig. 3).[10] The expression of *Ostβ*, *Bsep* and *Cyp7α1*, three well known FXR target genes, was therefore investigated by quantitative RT-PCR using RNA from HepG2 cells stimulated with these compounds. The results demonstrated that all compounds induced *Ostβ* with an activity of 20–40% compared to 1 μM of INT-747(**1**). *Cyp7α1* was down-regulated by all derivatives tested in dose-dependent manner, with ***s*(±)24** resulting the most effective compound. While compounds ***s*(±)24** and ***s*(±)38** do not induce *Bsep*, the compound ***s*(±)37** induced *Bsep* in dose-dependent manner.

2.4. Chemistry

The general synthetic strategy for the preparation of all the compounds is outlined in Scheme 1 and involved the condensation reaction between the appropriate 1-substituted-3-methyl-5-aminopyrazole **46** and an aryl aldehyde **47**, carried out in refluxing toluene with concomitant water removal. The intermediate 2,4-dihydro-3*H*-pyrazol-5-imines, thus formed, were then treated with 2-mercaptopropanoic acid to afford the title 1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-ones **8–14, 17–29, 33–45**.[25]

Since two chiral centers are present in the structure of the final compounds it was expected to obtain them as mixture of two racemic couples. Medium pressure chromatography of the crude reaction mixtures allowed us in most cases, to obtain the less polar racemic couples ***a*(±)8–14, *a*(±)17–29, *a*(±)33–41** and ***a*(±)45**, endowed with an *anti* disposition of the substituents (for the assignment, see below), and the more polar ones, ***s*(±)8–14, *s*(±)17–29, *s*(±)33–39**, and ***s*(±)45** showing a *syn* disposition of the substituents. The separation was not possible for the compounds **42–44** which were therefore tested as a mixture of four diastereoisomers.

The compounds ***a*-** and ***s*-(±)13**, **14**, **27, 28** and **29**, characterized by an ester functionality were then submitted to an alkaline hydrolysis to afford the corresponding carboxylic acids ***a*-** and ***s*(±)15**, **16**, **30–32** (Schemes 2 and 3).

The precursors 1-substituted-3-methyl-5-aminopyrazole **46a–l** were prepared in high yields following a known procedure involving the reaction of 3-aminocrotononitrile (**48**) with the appropriate arylhydrazine **49** (Scheme 4).[26] The commercial unavailable arylhydrazines **49d–l** were obtained according to a known protocol[27] by reduction with stannous chloride of the corresponding diazonium salts, in turn prepared starting from the corresponding anilines.

The 4-aryl-substituted benzaldehydes **47b–m**, needed for the preparation of the final compounds ***a*-** and ***s*(±)-9–14**,**-17–22** were synthesized in high yields, by a Suzuki coupling reaction protocol starting from 4-bromobenzaldehyde (**50**) and the appropriate aryl boronic acid **51** (Scheme 5).

The reaction of 4-fluorobenzaldehyde (**52**) with the monosubstituted phenols **53a–f**, pre-treated with potassium carbonate,

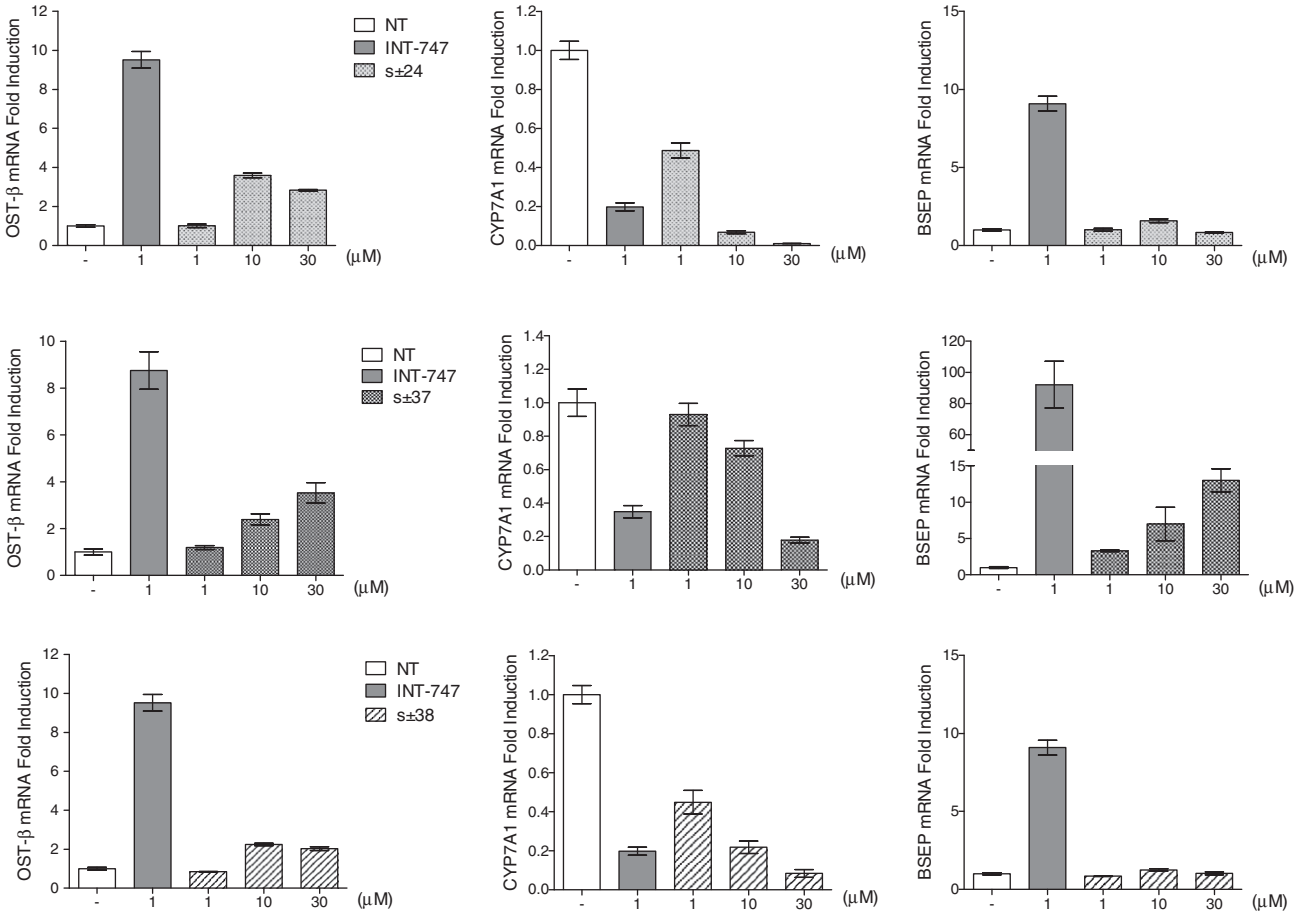
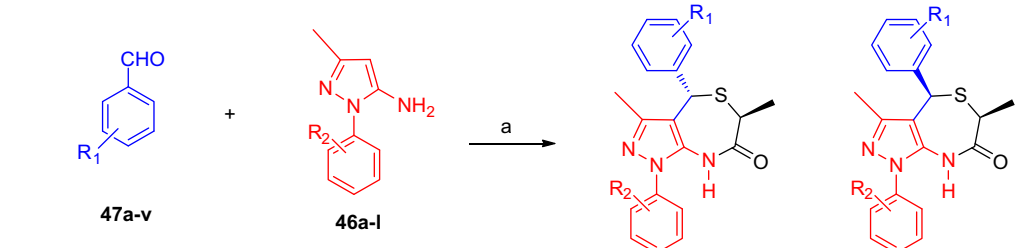


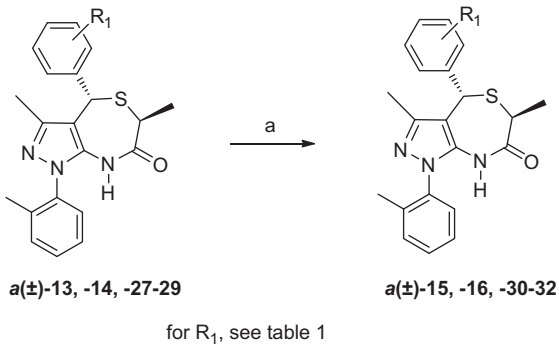
Figure 3. Regulation of *Ostβ*, *Bsep* and *Cyp7α1* genes by ***s*(±)24**, ***s*(±)37** and ***s*(±)38** assessed by quantitative RT-PCR. The results show mean ± SD of triplicate samples from a representative experiment of three performed.



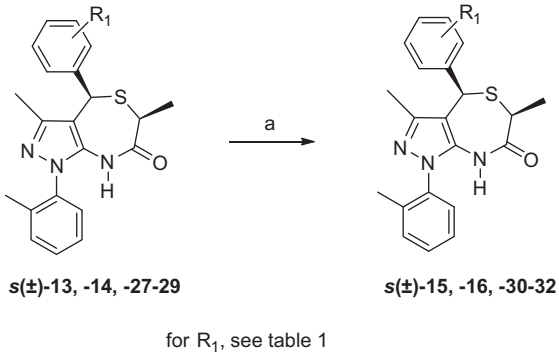
a: R_1 = 4-phenyl
b: R_1 = 4-(2-methoxyphenyl)
c: R_1 = 4-(3-methoxyphenyl)
d: R_1 = 4-(4-methoxyphenyl)
e: R_1 = 4-(3-ethoxyphenyl)
f: R_1 = 4-(3-CO_2CH_3phenyl)
g: R_1 = 4-(4-CO_2CH_3phenyl)
h: R_1 = 4-(*t*-styryl)
i: R_1 = 4-(2-thienyl)
j: R_1 = 4-(3-thienyl)
k: R_1 = 4-(2-furanyl)
l: R_1 = 4-(3-furanyl)
m: R_1 = 4-(2-benzofuranyl)
n: R_1 = 4-phenoxy
o: R_1 = 4-(2-methoxyphenoxy)
p: R_1 = 4-(3-methoxyphenoxy)
q: R_1 = 4-(4-methoxyphenoxy)
r: R_1 = 4-(2-CO_2CH_3phenoxy)
s: R_1 = 4-(3-CO_2CH_3phenoxy)
t: R_1 = 4-(4-CO_2CH_3phenoxy)
u: R_1 = 4-benzyloxy
v: R_1 = 3-methoxy-4-phenoxy

a: R_2 = 2-CH_3
b: R_2 = 3-CH_3
c: R_2 = 4-CH_3
d: R_2 = 2-C_2H_5
e: R_2 = 2-*n*C_3H_7
f: R_2 = 2-*i*C_3H_7
g: R_2 = 2-CF_3
h: R_2 = 2-F
i: R_2 = 2-Cl
j: R_2 = 2-Br
k: R_2 = 2-I
l: R_2 = 2-OCH_3

Scheme 1. Synthesis of 1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-ones, **a(±)**- and **s(±) 8–14, -17–29, -33–45**. Reagents and conditions: (a) (i) toluene, reflux; (ii) 2-mercaptopropanoic acid, toluene, reflux; (iii) medium pressure liquid chromatography.



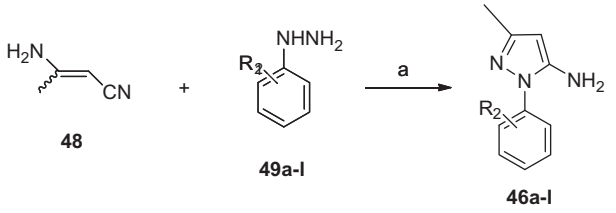
Scheme 2. Synthesis of 1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-ones, ***a*(±)15, 16, 30–32**. Reagents and conditions: (a) 0.1 N NaOH, THF, rt.



Scheme 3. Synthesis of 1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-ones, ***s*(±)15, 16, 30–32**. Reagents and conditions: (a) 0.1 N NaOH, THF, rt.

afforded, under microwave irradiation, 4-aryloxy-substituted benzaldehydes **44o–t** used in the preparation of the final compounds ***a***- and ***s*(±)24–29.** (Scheme 6).

The assignment of the relative configurations to all the synthesized racemic mixtures started from the spectroscopic analysis of the two racemic couples, ***a*(±)23** and ***s*(±)23.** In the less polar couple ***a*(±)23**, strong NOE occurs between H-6 (quartet at 3.20 ppm) and the aromatic protons of the aryl moiety at C-4 position, whereas the NOESY spectrum of the more polar diastereoisomer ***s*(±)23** shows NOE between H-4 (singlet at 5.63 ppm) and H-6 (quartet at 3.81 ppm), suggesting a *syn* disposition between these two hydrogens. These data allowed us to suppose that in ***s*(±)23** the seven-member ring adopt such a conformation as the phenoxyphenyl moiety and the C-6 methyl are both oriented in equatorial position (graphically represented by conformer **A** in Figure 4). The absence of cross peak between the C-6 methyl and the aryl group suggested that the energetically unfavourable conformation locating both these substituents in pseudo-axial orientation did not exist (conformer **B** in Fig. 4). In the case of ***a*(±)23**, the cross peak occurring between the hydrogen at C-6 position and the aromatic protons of the aryl moiety at C-4 position suggested that the preferred azepine ring conformation, graphically depicted by



a: R_2= 2-CH_3;
b: R_2 = 3-CH_3;
c: R_2= 4-CH_3;
d: R_2 = 2-C_2H_5;
e: R_2 = 2-*n*C_3H_7;
f: R_2 = 2-*i*C_3H_7;
g: R_2 = 2-CF_3;
h: R_2 = 2-F;
i: R_2 = 2-Cl;
j: R_2 = 2-Br;
k: R_2 = 2-I;
l: R_2 = 2-OCH_3.

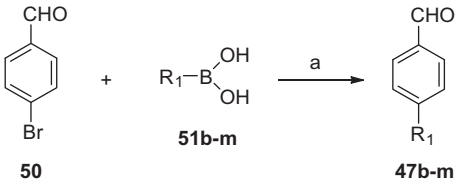
Scheme 4. Synthesis of 3-methyl-5 amino1*H*-pyrazoles, **46a–l**. Reagents and conditions: (a) 12 N HCl, H_2O, reflux.

conformer **A** in Figure 5, was that orienting the aryl moiety and 6-CH in pseudo-axial position, whereas it was unfavourable the conformation locating the same moieties in pseudo-equatorial disposition (conformer **B** in Fig. 5).

By moving to compare the H-4 and H-6 chemical shifts in ***a*-** and ***s*(±)23** we noted remarkable differences: 3.22 ppm in ***a*(±)23** versus 3.81 ppm in ***s*(±)23** for H-6, and 5.21 ppm versus 5.63 ppm for H-4 in ***a*(±)23** and ***s*(±)23**, respectively. The analysis of the ^{1}H NMR data of all the other final compounds allows to emerge as a rule for a specific molecule that both H-4 and H-6 are always more deshielded in the more polar pair, as observed in the more polar ***s*(±)23**. According to this evidence the *anti* relative configuration was assigned to all the less polar pairs and the *syn* one to the more polar couples.

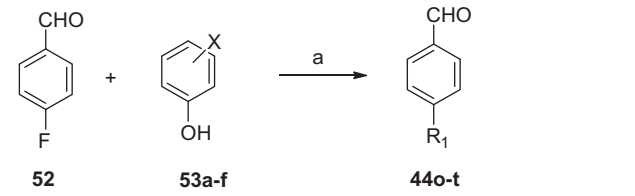
2.5. Binding mode assessment

The analysis of the results reported in Tables 1 and 2 allows to draw a preliminary scheme of SAR as composed of two regions harboring rings A and B (Fig. 6). The first region, accommodating



b: R_1 = 4-(2-methoxyphenyl)
c: R_1 = 4-(3-methoxyphenyl)
d: R_1 = 4-(4-methoxyphenyl)
e: R_1 = 4-(3-ethoxyphenyl)
f: R_1 = 4-(3-CO_2CH_3phenyl)
g: R_1 = 4-(4-CO_2CH_3phenyl)
h: R_1 = 4-(*E*-styryl)
i: R_1 = 4-(2-thienyl)
j: R_1 = 4-(3-thienyl)
k: R_1 = 4-(2-furanyl)
l: R_1 = 4-(3-furanyl)
m: R_1 = 4-(2-benzofuranyl)

Scheme 5. Synthesis of the 4-(aryl)benzaldeydes, **47b–m**. Reagents and conditions: (a) $NaHCO_3$, H_2O, tetrakis(triphenylphosphine)palladium(0), toluene–EtOH 1:1, reflux.



a: X = 2-OCH_3
b: X = 3-OCH_3
c: X = 4-OCH_3
d: X = 2-$CO_2C_2H_5$
e: X = 3-CO_2CH_3
f: X = 4-CO_2CH_3
o: R_1 = 4-(2-methoxyphenoxy)
p: R_1 = 4-(3-methoxyphenoxy)
q: R_1 = 4-(4-methoxyphenoxy)
r: R_1 = 4-(2-CO_2CH_3phenoxy)
s: R_1 = 4-(3-CO_2CH_3phenoxy)
t: R_1 = 4-(4-CO_2CH_3phenoxy)

Scheme 6. Synthesis of 4-(aryloxy)benzaldehydes, **44o–t**. Reagents: (a) K_2CO_3, DMF, microwave irradiation.

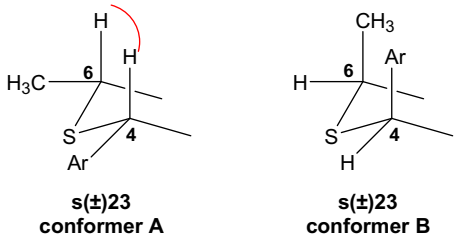


Figure 4. Graphical representation of the conformations of ***s*(±)23**.

ring A, should be envisaged as large enough to lodge a phenoxyphenyl moiety and composed of aromatic and/or hydrophobic residues. At this regard, it is worth noting that the presence of acidic or ester groups on ring A substituents [***s*(±)13–16, *s*(±)27–32**] is fully detrimental for the activity. Overall, the occupancy of this region affects both the activity and the efficacy of the compounds. Conversely, the second region lodging ring B seems to mostly influence the efficacy of the compounds rather than their activity. These data suggest that the ring A is the key-player with respect to the ring B, giving the higher contribution to the driving force that govern the binding process. Again, SAR of the explored substituents pinpoints the hydrophobic and/or aromatic character of residues facing this part of the binding site.

The scheme of Figure 6 was next used as criteria to select a putative binding mode of the hit compound **7** and the optimized lead ***s*(±)38** that could be compliant with the properties of regions 1 and 2. The top scored poses obtained by docking experiments of compounds **7** and ***s*(±)38** with the FXR structure (pdb code: 1OSV[10]) are shown in Figure 7. Unfortunately, these poses (as well as all of the remaining low scored poses) were not in agreement with the reported SAR scheme, with the major point of disagreement being the presence of Arg328 in the supposed hydrophobic region 1.

Thus, although the scoring function of docking experiments was successful in sorting out true FXR binders in the virtual screening campaign, it did not propose a compelling binding pose for them. The fallacy to predict true binding modes in docking screens has been recently debated.[28] In that paper, the authors argued that in the absence of a correct binding pose for hit compounds, a skeptic might well infer that the discovery is serendipitous.

Although we have inconsistent data between the SAR scheme and docking results, we deem that the discovery of **7** as FXR ligand may still not to be considered serendipitous, given the following consideration. Our virtual screening is not fully dependent on a structural hypothesis generated from docking experiments, rather

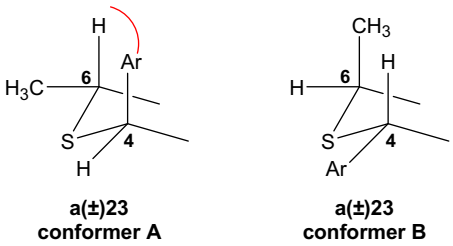


Figure 5. Graphical representation of the conformations of ***a*(±)23**.

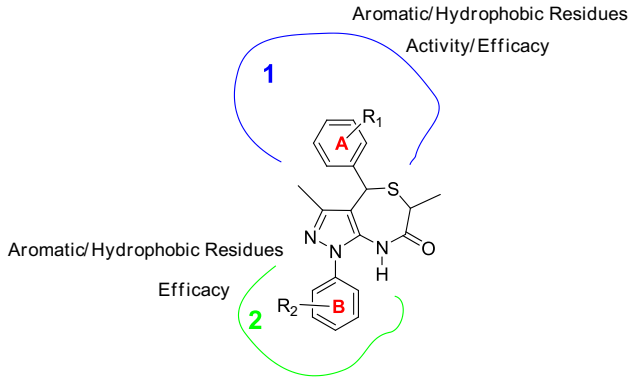


Figure 6. SAR analysis observations. The phenyl ring A lies in an hydrophobic/aromatic region (1) affecting the activity and efficacy of the compounds. Ring B is lodged into a second aromatic/hydrophobic region (2) influencing the efficacy of the compounds.

it implements the combination of a pharmacophoric hypothesis with an energetical hypothesis from the scoring function of docking experiments. The 39.8% hit rate resulting from such a combination is high, sustaining the robustness of this strategy as well as the validity of the employed scoring function.

Still, the question is why the proposed binding pose does not agree with the SAR scheme. One explanation could be that the adopted semi-rigid docking procedure (ligand free and protein rigid) neglects induced-fit aspects that are pivotal for the correct positioning of the ligand within the binding cleft of the receptor. At this regard, molecular dynamics[29,30] and crystallization studies[10,11,13,16–19] support the high flexibility of FXR ligand binding domain.

The fact that compound **7** is successfully identified as FXR binder underlines that a correct prediction of the binding pose is not mandatory for a successful virtual screening, albeit it is important for an efficient hit to lead and lead to candidate optimization. Although solving a crystal structure of the complex should be the method of choice to determine the binding pose, it is not always feasible or not readily accessible. In such a case, the development of SAR schemes may allow to get clues on the right binding pose adopted by hit compounds into the target receptor.

3. Conclusion

In an effort to identify novel non-steroidal FXR agonists, a sequential focused virtual screening approach was adopted to search within DrugLike (2 million of compounds) and the LeadLike (1 million of compounds) subsets of the ZINC 8 database. By biologically testing only 117 selected compounds, 34 compounds were successfully identified as FXR ligands, showing activity below a concentration of 20 μM. In particular, 4-(2,4-dimethoxyphenyl)-3,6-dimethyl-1-(2-tolyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one (**7**, EC_{50} = 10.5 μM, efficacy = 63%) was selected as a new scaffold for an initial hit-to-lead optimization focused to explore substitution patterns of rings A and B of **7**. Briefly, the replacement of the 2,4-dimethoxyphenyl group of **7** by a biphenyl or 4-phenoxyphenyl moiety increased the potency of the corresponding derivatives, whereas the introduction at the *ortho*-position of the *N*-phenyl ring of alkyl groups bulkier than the methyl one influenced positively the efficacy of the resulting derivatives. The resulting SAR scheme was instrumental to assess a putative

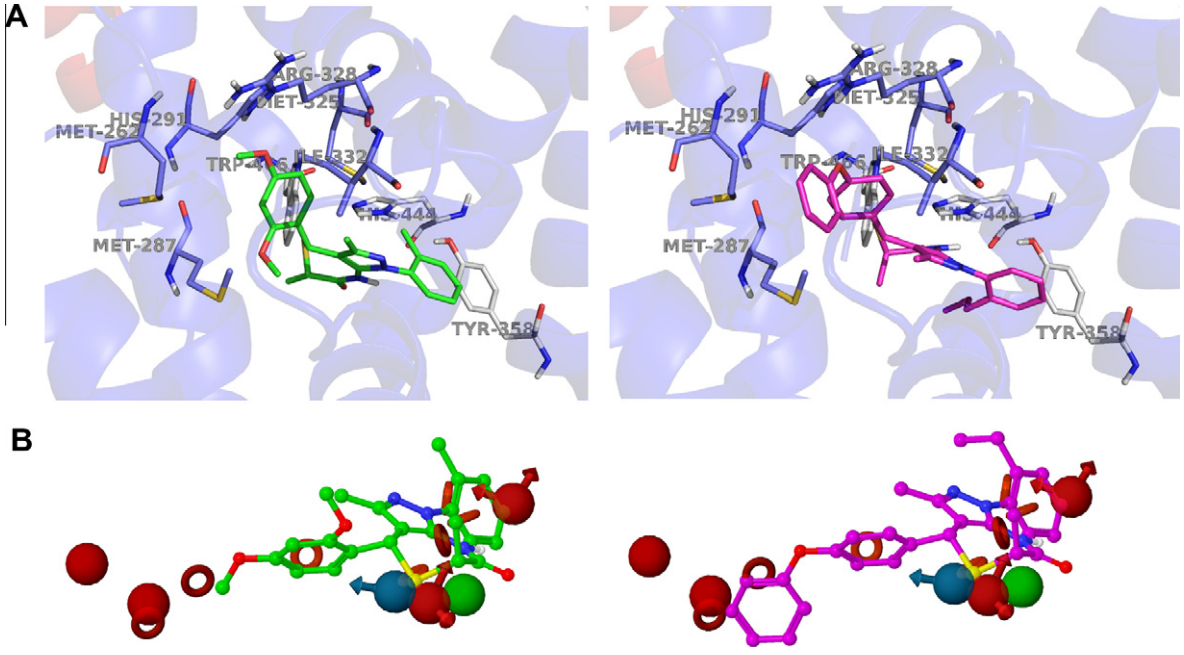


Figure 7. (A) Ligand interactions of **7** (left) and **s(±)38** (right). The docking poses in the 1OSV X-ray complex of FXR with the main residues involved in the interaction with the phenyl moiety are colored in blue and the trigger triad shown in white. The coactivator helix is shown in red ribbon. (B) Pharmacophoric poses of **7** (left) and **s(±)38** (right), with snapshot taken with a common view with the docking poses. The regions occupied by ring A and B are the same as observed in A.

binding mode of the hit compound **7** and ***s*(±)38** into the binding cleft of FXR. Unfortunately, no binding pose was found in agreement with the SAR scheme, prompting for further in-depth studies.

In conclusion, we got the identification of *syn*-(±)-4-[(4-(2-methoxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [***s*(±)24**], *syn*-(±)-4-(4-phenoxyphenyl)-3,6-dimethyl-1-(2-ethylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [***s*(±)37**] and *syn*-(±)-4-(4-(phenoxyphenyl)-3,6-dimethyl-1-(2-*n*propylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [***s*(±)38**], three derivatives exhibiting low micromolar range of potency (10-fold higher than the initial hit) and full efficacy.

Additional studies are ongoing with the aim of further optimize this series of compounds, also devising compounds combining fruitful substitutions of rings A and B. Likewise, further work will be directed to gain insights into the putative binding pose of this series of compounds into the ligand binding cleft of FXR.

4. Experimental section

4.1. General methods

Melting points were determined by the capillary method on a Büchi 535 electrothermal apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were taken on a Bruker AC 200 or Bruker AC 400 spectrometers as solutions in $CDCl_3$ unless otherwise indicated. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and bs (broad). Flash chromatography was performed on Merck silica gel (0.040–0.063 mm). Medium pressure chromatography (MPC) was performed on Merck LiChroprep Si 60 Lobar columns. All target compounds possessed acceptable purity as verified by HPLC (see Supplementary data).

4.2. General procedure for the synthesis of 1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-ones 8–14, 17–29, 33–45

The appropriate 1-substituted-3-methyl-5-aminopyrazole **46** (1.0 mmol) and the aldehyde **47** (1.0 mmol) were heated under reflux in toluene (50 mL) for 7–9 hours; during this period produced water was removed with the aid of a Dean-Stark apparatus. The reaction mixture was cooled, 2-mercaptopropanoic acid (1.0 mmol) was added and the mixture heated again at reflux for 12 hours. The solvent was removed in vacuo and the residue submitted to medium pressure liquid chromatography. Elution by light petroleum–ethyl acetate mixtures afforded the desired compounds.

4.2.1. *anti*-(±)-4-Biphenyl-4-yl-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)8]

Light petroleum–ethyl acetate (80:20), yield 32%, white solid, mp 193–194 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (3H, d, J = 7.2 Hz, 6-CH_3), 1.98 (3H, s, 3-CH_3), 2.18 (3H, s, 2-CH_3), 3.28 (1H, q, J = 7.2 Hz, 6-CH), 5.22 (1H, s, 4-CH), 7.13 (1H, s, NH), 7.34–7.65 (13H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.80, 16.37, 17.30, 36.25, 42.50, 104.87, 126.97, 127.24, 127.49, 128.36, 128.54, 128.83, 130.52, 131.72, 135.28, 136.24, 136.99, 140.23, 140.30, 140.99, 147.84, 172.16.

4.2.2. *syn*-(±)-4-Biphenyl-4-yl-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)8]

Light petroleum–ethyl acetate (80:20), yield 33%, white solid, mp 232–235 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (3H, d, J = 7.2 Hz, 6-CH_3), 1.92 (3H, s, 3-CH_3), 2.13 (3H, s, 2-CH_3), 3.85 (1H, q, J = 7.2 Hz, 6-CH), 5.68 (1H, s, 4-CH), 7.00 (1H, s, NH), 7.33–7.61 (13H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.36, 15.24, 17.23, 39.01, 45.15, 108.43, 127.02, 127.28, 127.46, 128.25, 128.61, 128.76, 130.28, 131.53, 135.34, 135.58, 136.59, 138.25, 140.41, 140.84, 148.15, 172.65; Anal. Calcd for $C_{27}H_{25}N_3OS$: C, 73.77; H, 5.73; N, 9.56; O, 3.64; S, 7.29. Found: C, 73.98; H, 5.73; N, 9.56; S, 7.30.

4.2.3. *anti*-(±)-4-(2′-Methoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)9]

Light petroleum–ethyl acetate (65:35), yield 31%, white solid, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 2.00 (3H, s, 2-CH_3), 2.12 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 3.80 (3H, s, OCH_3), 5.25 (1H, s, 4-CH), 7.0–7.55 (13H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.86, 16.44, 17.31, 36.37, 42.61, 55.54, 105.05, 111.35, 120.92, 127.52, 127.60, 128.58, 128.84, 129.69, 130.55, 130.83, 131.74, 135.30, 136.25, 137.05, 137.78, 140.32, 147.93, 156.47, 172.24.

4.2.4. *syn*-(±)-4-(2′-Methoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)9]

Light petroleum–ethyl acetate (65:35), yield 31%, white solid, mp 190–193 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50 (3H, d, J = 7.2 Hz, 6-CH_3), 1.95 (3H, s, 2-CH_3), 2.10 (3H, s, 3-CH_3), 3.75 (4H, m, 6-CH and OCH_3), 5.70 (1H, s, 4-CH), 7.0–7.55 (13H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.38, 15.22, 17.27, 38.95, 45.18, 55.53, 60.4, 108.76, 111.30, 120.87, 127.30, 127.81, 128.29, 128.78, 129.90, 130.30, 130.82, 131.55, 135.39, 135.59, 136.61, 137.75, 138.23, 148.24, 156.45, 172.79; Anal. Calcd for $C_{28}H_{27}N_3OS$: C, 71.61; H, 5.80; N, 8.95; O, 6.81; S, 6.83. Found: C, 71.86; H, 5.82; N, 8.94; S, 6.84.

4.2.5. *anti*-(±)-4-(3′-Methoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)10]

Light petroleum–ethyl acetate (65:35), yield 37%, white solid, mp 147–149 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 2.00 (3H, s, 2-CH_3), 2.20 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 3.82 (3H, s, OCH_3), 5.23 (1H, s, 4-CH), 6.97 (1H, s, NH), 7.0–7.68 (12H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.75, 16.34, 17.28, 36.27, 42.49, 55.29, 104.91, 112.74, 112.90, 119.48, 127.30, 127.49, 128.32, 128.54, 129.83, 130.54, 131.72, 135.21, 136.26, 137.01, 140.21, 141.13, 141.76, 147.83, 159.98, 172.24.

4.2.6. *syn*-(±)-4-(3′-Methoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)10]

Light petroleum–ethyl acetate (65:35), yield 35%, white solid, mp 130–135 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (3H, d, J = 7.2 Hz, 6-CH_3), 1.85 (3H, s, 2-CH_3), 2.10 (3H, s, 3-CH_3), 3.80 (4H, m, 6-CH and OCH_3), 5.70 (1H, s, 4-CH), 6.90 (1H, m, aromatic), 7.10 (1H, s, NH), 7.12–7.68 (11H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.21, 15.15, 17.21, 38.96, 45.03, 55.24, 108.62, 112.74, 112.84, 119.51, 127.21, 127.50, 128.287, 128.55, 129.75, 130.29, 131.48, 135.36, 135.50, 136.60, 138.36, 140.71, 141.90, 148.07, 159.90, 173.02; Anal. Calcd for $C_{28}H_{27}N_3OS$: C, 71.61; H, 5.80; N, 8.95; O, 6.81; S, 6.83. Found: C, 71.88; H, 5.82; N, 8.93; S, 6.84.

4.2.7. *anti*-(±)-4-(4′-Methoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)11]

Light petroleum–ethyl acetate (65:35), yield 28%, white solid, mp 218–220 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2,

6-CH_3), 1.90 (3H, s, 2-CH_3), 2.15 (3H, s, 3-CH_3), 3.25 (3H, s, OCH_3), 3.80 (1H, q, J = 7.2 Hz, 6-CH), 5.25 (1H, s, 4-CH), 6.90 (2H, m, aromatics), 7.02 (1H, s, NH), 7.25–7.55 (10H, m, aromatics); ^{13}C NMR (CDCl3, 1400 MHz) δ 12.79, 16.39, 17.30, 29.69, 36.28, 42.52, 55.35, 104.974, 114.29, 126.77, 127.52, 128.02, 128.36, 128.56, 130.57, 131.75, 132.77, 135.26, 136.26, 137.03, 139.94, 140.27, 147.86, 159.32, 172.20.

4.2.8. *syn*-(±)-4-(4′-Methoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)11]

Light petroleum–ethyl acetate (65:35), yield 26%, white solid, mp 112–115 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (3H, d, J = 7.2, 6-CH_3), 1.90 (3H, s, 2-CH_3), 2.12 (3H, s, 3-CH_3), 3.85 (4H, m, 6-CH and OCH_3), 5.70 (1H, s, 4-CH), 6.93 (2H, m, aromatics), 7.02 (1H, s, NH), 7.25–7.55 (10H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.31, 15.26, 17.27, 29.70, 39.07, 45.18, 55.34, 108.63, 114.24, 127.04, 127.34, 128.07, 128.33, 128.60, 130.41, 131.59, 132.94, 135.35, 135.532, 136.665, 137.47, 140.51, 148.17, 159.29, 172.79; Anal. Calcd for $C_{28}H_{27}N_3OS$: C, 71.61; H, 5.80; N, 8.95; O, 6.81; S, 6.83. Found: C, 71.60; H, 5.79; N, 8.93; S, 6.84.

4.2.9. *anti*-(±)-4-(3′-Ethoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)12]

Light petroleum–ethyl acetate (70:30), yield 32%, white solid, mp 96–100 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 1.48 (3H, t, J = 7.1 Hz, OCH_2CH_3), 1.97 (3H, s, 2-CH_3), 2.20 (3H, s, 3-CH_3), 3.26 (1H, q, J = 7.2 Hz, 6-CH), 4.17 (2H, q, J = 7.1 Hz, OCH_2CH_3), 5.23 (1H, s, 4-CH), 6.81 (m, 1H, aromatic), 7.13–7.60 (m, 12H, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.76, 14.83, 16.29, 17.27, 36.20, 42.45, 63.41, 104.92, 113.28, 119.29, 127.23, 127.38, 128.26, 128.52, 129.77, 130.40, 131.62, 135.29, 136.21, 136.92, 140.17, 141.13, 141.66, 147.81, 159.29, 172.38.

4.2.10. *syn*-(±)-4-(3′-Ethoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)12]

Light petroleum–ethyl acetate (70:30), yield 31%, white solid, mp 103–107 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (6H, m, 6-CH_3 and OCH_2CH_3), 1.95 (3H, s, 2-CH_3), 2.13 (3H, s, 3-CH_3), 3.79 (1H, q, J = 7.2 Hz, 6-CH), 4.17 (2H, q, J = 7.1 Hz, OCH_2CH_3), 5.62 (1H, s, 4-CH), 6.81 (1H, m, aromatic), 7.13 (1H, s, NH), 7.14–7.60 (11H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.28, 14.83, 15.16, 17.22, 38.89, 45.02, 63.40, 108.52, 113.29, 119.35, 127.12, 127.44, 128.25, 128.54, 129.71, 130.12, 131.40, 135.39, 135.62, 136.56, 138.41, 140.66, 141.84, 148.05, 159.23, 173.03; Anal. Calcd for $C_{29}H_{29}N_3OS$: C, 72.02; H, 6.04; N, 8.69; O, 6.62; S, 6.63. Found: C, 72.28; H, 6.05; N, 8.68; S, 6.63.

4.2.11. *anti*-(±)-4-(3′-Carbomethoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)13]

Light petroleum–ethyl acetate (65:35), yield 35%, white solid, mp 198–200 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 1.96 (3H, s, 2-CH_3), 2.16 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 3.96 (3H, s, CO_2CH_3), 5.20 (1H, s, 4-CH), 7.01 (1H, s, NH), 7.35–7.47 (6H, m, aromatics), 7.54 (1H, t, J = 7.7 Hz, aromatic), 7.60 (2H, d, J = 8.4 Hz, aromatics), 7.81 (1H, m, aromatic), 8.04 (1H, m, aromatic), 8.31 (1H, m, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.80, 16.38, 17.30, 36.27, 42.48, 52.23, 104.77, 127.35, 127.53, 128.19, 128.51, 128.96, 130.57, 130.77, 131.36, 131.75, 135.26, 136.28, 136.99, 139.30, 140.59, 141.61, 147.80, 166.96, 172.07.

4.2.12. *syn*-(±)-4-(3′-Carbomethoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)13]

Light petroleum–ethyl acetate (65:35), yield 31%, white solid, mp 184–186 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.47 (3H, d, J = 7.2 Hz, 6-CH_3), 1.90 (3H, s, 2-CH_3), 2.11 (3H, s, 3-CH_3), 3.86 (1H, q, J = 7.2 Hz, 6-CH), 3.96 (3H, s, CO_2CH_3), 5.71 (1H, s, 4-CH), 6.97 (1H, s, NH), 7.30–7.46 (6H, m, aromatics), 7.52 (1H, t, J = 7.7 Hz, aromatic), 7.60 (2H, d, J = 8.4 Hz, aromatic), 7.77 (1H, m, aromatic), 8.03 (1H, m, aromatic), 8.27 (1H, m, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.34, 15.25, 17.25, 39.06, 45.13, 52.21, 108.38, 127.33, 127.54, 128.19, 128.26, 128.53, 128.78, 128.91, 130.36, 130.73, 131.40, 131.59, 135. 42, 135.49, 136.62, 138.86, 139.80, 140.70, 148.10, 166.96, 172.57; Anal. Calcd for $C_{29}H_{27}N_3O_3S$: C, 70.00; H, 5.47; N, 8.44; O, 9.65; S, 6.44. Found: C, 69.88; H, 5.48; N, 8.45; S, 6.45.

4.2.13. *anti*-(±)-4-(4′-Carbomethoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)14]

Light petroleum–ethyl acetate (60:40), yield 34%, white solid, mp 220–223 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (3H, d, J = 7.2 Hz, 6-CH_3), 1.98 (3H, s, 2-CH_3), 2.18 (3H, s, 3-CH_3), 3.27 (1H, q, J = 7.1 Hz, 6-CH), 3.97 (3H, s, CO_2CH_3), 5.22 (1H, s, 4-CH), 7.07 (1H, s, NH), 7.28–7.43 (6H, m, aromatics), 7.66 (2H, d, J = 8.3 Hz, aromatics), 7.71 (2H, d, J = 8.3 Hz, aromatics), 8.14 (2H, d, J = 8.3 Hz, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 12.77, 16.38, 17.30, 36.30, 42.46, 52.16, 104.73, 126.91, 127.47 127.55, 128.53, 129.12, 130.17, 130.64, 131.79, 135.14, 136.32, 137.00, 139.16, 142.02, 144.65, 147.77, 166.91, 172.02.

4.2.14. *syn*-(±)-4-(4′-Carbomethoxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)14]

Light petroleum–ethyl acetate (60:40), yield 32%, white solid, mp 201–204 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.47 (3H, d, J = 7.1 Hz, 6-CH_3), 1.91 (3H, s, 2-CH_3), 2.12 (3H, s, 3-CH_3), 3.84 (1H, q, J = 7.1 Hz, 6-CH), 3.95 (3H, s, CO_2CH_3), 5.67 (1H, s, 4-CH), 6.89 (1H, s, NH), 7.32–7.46 (6H, m, aromatics), 7.61 (2H, d, J = 8.3 Hz, aromatics), 7.66 (2H, d, J = 8.3 Hz, aromatics), 8.11 (2H, d, J = 8.3 Hz, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 13.30, 15.25, 17.25, 39.11, 45.11, 52.14, 108.38, 126.95, 127.39 127.68, 128.26, 128.79, 129.08, 130.13, 130.49, 131.64, 135.27, 135.51, 136.61, 139.23, 139.70, 144.78, 148.06, 166.91, 172.50; Anal. Calcd for $C_{29}H_{27}N_3O_3S$: C, 70.00; H, 5.47; N, 8.44; O, 9.65; S, 6.44. Found: C, 70.01; H, 5.48; N, 8.43; S, 6.45.

4.2.15. *anti*-(±)-4-{4-[(*E*)-2-Phenylvinyl]phenyl}-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)17]

Light petroleum–ethyl acetate (80:20), yield 29%, white solid, mp 133–136 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 2.00 (3H, s, 2-CH_3), 2.20 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.20 (1H, s, 4-CH), 7.05–7.55 (16H, m, *CH*=*CH*, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.73, 16.33, 17.26, 36.17, 42.55, 104.78, 126.49, 126.68, 127.46, 127.79, 128.24, 128.50, 128.67, 129.22, 130.50, 131.69, 135.23, 136.17, 136.64, 137.06, 141.26, 147.79, 172.13.

4.2.16. *syn*-(±)-4-{4-[(*E*)-2-Phenylvinyl]phenyl}-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)17]

Light petroleum–ethyl acetate (80:20); yield 30%, white solid, mp 203–206 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (3H, d, J = 7.2 Hz, 6-CH_3), 1.90 (3H, s, 2-CH_3), 2.12 (3H, s, 3-CH_3), 3.85

(1H, q, J = 7.2 Hz, 6-CH), 5.65 (1H, s, 4-CH), 7.10 (1H, s, NH), 7.25–7.55 (15H, m, C*H*=C*H* and aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.29, 15.21, 17.21, 38.94, 45.22, 108.38, 126.48, 126.85, 127.19, 127.69, 127.89, 128.24, 128.53, 128.63, 129.11, 130.21, 131.45, 135.33, 135.54, 136.58, 137.06, 138.45, 148.07, 172.80; Anal. Calcd for $C_{29}H_{27}N_3OS$: C, 74.81; H, 5.84; N, 9.02; O, 3.44; S, 6.89. Found: C, 74.82; H, 5.84; N, 9.03; S, 6.90.

4.2.17. *anti*-(±)-4-[(4-(2-Thienyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)18]

Light petroleum–ethyl acetate (80:20), yield 30%, white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 1.99 (3H, s, 2-CH_3), 2.20 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.21 (1H, s, 4-CH), 7.10 (1H, m, aromatic), 7.20 (1H, s, NH), 7.25–7.50 (8H, m, aromatics), 7.60 (2H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.72, 16.31, 17.25, 36.21, 42.47, 104.73, 123.28, 125.05, 125.98, 127.45, 128.08, 128.43, 128.50, 130.48, 131.68, 133.66, 135.25, 136.20, 136.94, 141.13, 143.52, 147.78, 172.19.

4.2.18. *syn*-(±)-4-[(4-(2-Thienyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)18]

Light petroleum–ethyl acetate (80:20), yield 31%, white solid, mp 224–227 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50 (3H, d, J = 7.2 Hz, 6-CH_3), 1.91 (3H, s, 2-CH_3), 2.12 (3H, s, 3-CH_3), 3.79 (1H, q, J = 7.2 Hz, 6-CH), 5.60 (1H, s, 4-CH), 7.04 (1H, s, NH), 7.24–7.46 (7H m, aromatics), 7.54 (1H, d, J = 8.1 Hz, aromatic), 7.60 (1H, d, J = 8.1 Hz, aromatic), 7.85 (2H, d, J = 8.2 Hz, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.28, 15.19, 17.20, 38.97, 45.11, 108.354, 123.29, 125.00, 126.19, 127.23, 128.01, 128.23, 128.721, 130.25, 131.49, 134.08, 135.33, 135.52, 136.58, 138.37, 143.63, 148.08, 172.82; Anal. Calcd for $C_{25}H_{23}N_3OS_2$: C, 67.39; H, 5.20; N, 9.43; O, 3.59; S, 14.39. Found: C, 67.19; H, 5.19; N, 9.43; S, 14.40.

4.2.19. *anti*-(±)-4-[(4-(3-Thienyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)19]

Light petroleum–ethyl acetate (80:20), yield 35%, white solid, mp 227–229 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.27 (3H, d, J = 7.2 Hz, 6-CH_3), 1.97 (3H, s, 2-CH_3), 2.18 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.18 (1H, s, 4-CH), 7.10 (s, 1H, NH), 7.25–7.50 (9H, m, aromatics), 7.60 (2H, d, J = 8.2 Hz, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.74, 16.33, 17.28, 36.23, 42.51, 104.86, 120.52, 126.08, 126.45, 126.55, 127.49, 128.40, 128.53, 130.53, 131.72, 135.03, 135.23, 136.25, 136.98, 140.77, 141.45, 147.81, 172.19.

4.2.20. *syn*-(±)-4-[(4-(3-Thienyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)19]

Light petroleum–ethyl acetate (80:20), yield 34%, white solid, mp 220–225 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (3H, d, J = 7.2 Hz, 6-CH_3), 1.91 (3H, s, 2-CH_3), 2.12 (3H, s, 3-CH_3), 3.84 (1H, q, J = 7.2 Hz, 6-CH), 5.66 (1H, s, 4-CH), 7.00 (1H, s, NH), 7.25–7.48 (9H, m, aromatics), 7.60 (2H, d, J = 8.2 Hz, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.31, 15.24, 17.23, 39.02, 45.19, 108.40, 120.52, 126.16, 126.32, 126.75, 127.28, 128.265, 128.67, 130.30, 131.54, 135.37, 135.51, 136.61, 138.00, 141.60, 148.11, 172.67; Anal. Calcd for $C_{25}H_{23}N_3OS_2$: C, 67.39; H, 5.20; N, 9.43; O, 3.59; S, 14.39. Found: C, 67.28; H, 5.20; N, 9.44; S, 14.40.

4.2.21. *anti*-(±)-4-[(4-(2-Furanyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)20]

Light petroleum–ethyl acetate (70:30), yield 21%, white solid, mp 118–121 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 1.96 (3H, s, 2-CH_3), 2.20 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.23 (1H, s, 4-CH), 6.50 (1H, m, aromatic), 6.73 (1H, m, aromatic), 7.25 (1H, s, NH), 7.27–7.52 (7H, m, aromatics), 7.70 (2H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.68, 16.27, 17.25, 36.19, 42.56, 104.80, 105.42, 111.71, 123.95, 127.44, 128.26, 128.52, 130.16, 130,49, 131.68, 135.23, 136.22, 136.96, 140.93, 142.28, 147.81, 153.33, 172.29.

4.2.22. *syn*-(±)-4-[(4-(2-Furanyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)20]

Light petroleum-ethyl acetate (70:30), yield 22%, white solid, mp 147–149 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (3H, d, J = 7.2 Hz, 6-CH_3), 1.96 (3H, s, 2-CH_3), 2.18 (3H, s, 3-CH_3), 3.83 (1H, q, J = 7.2 Hz, 6-CH), 5.70 (1H, s, 4-CH), 6.50 (m, 1H, aromatic), 6.73 (1H, m, aromatic), 7.25–7.50 (8H, m, aromatics and NH), 7.62 (2H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.19, 15.17, 17.20, 38.97, 45.20, 105.38, 108.46, 111.67, 124.10, 127.19, 128.27, 128.55, 130.25, 130,54, 131.46, 135.44, 136.61, 138.08, 142.21, 148.07, 153.38, 173.04; Anal. Calcd for $C_{25}H_{23}N_3O_2S$: C, 69.91; H, 5.40; N, 9.78; O, 7.45; S, 7.47. Found: C, 69.88; H, 5.42; N, 9.76; S, 7.45.

4.2.23. *anti*-(±)-4-[(4-(3-Furanyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)21]

Light petroleum-ethyl acetate (75:25), yield 23%, white solid, mp 223–226 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 1.92 (3H, s, 2-CH_3), 2.10 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.22 (1H, s, 4-CH), 6.75 (1H, s, aromatic), 7.20 (1H, s, NH), 7.25–7.50 (9H, m, aromatics), 7.75 (1H, s, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.71, 16.31, 17.26, 36.18, 42.49, 104.85, 108.62, 125.76, 125.98, 127.45, 128.37, 128.52, 130.50, 131.68, 135.21, 136.20, 136.96, 138.65, 140.63, 143.82, 147.80, 172.28.

4.2.24. *syn*-(±)-4-[(4-(3-Furanyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)21]

Light petroleum-ethyl acetate (75:25), yield 23%, white solid, mp 161–163 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50 (3H, d, J = 7.2 Hz, 6-CH_3), 1.88 (3H, s, 2-CH_3), 2.07 (3H, s, 3-CH_3), 3.76 (1H, q, J = 7.2 Hz, 6-CH), 5.63 (1H, s, 4-CH), 6.75 (1H, s, aromatic), 7.25–7.50 (10H, m, aromatics and NH), 7.75 (1H, s, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.25, 15.17, 17.21, 38.94, 45.14, 108.49, 108.68, 125.85, 126.16, 127.18, 128.26, 128.65, 130.21, 131.45, 132.12, 135.37, 135.53, 136.60, 137.86, 138.62, 143.71, 148.07, 173.07; Anal. Calcd for $C_{25}H_{23}N_3O_2S$: C, 69.91; H, 5.40; N, 9.78; O, 7.45; S, 7.47. Found: C, 69.92; H, 5.42; N, 9.77; S, 7.45.

4.2.25. *anti*-(±)-4-[(4-(2-Benzofuranyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)22]

Light petroleum-ethyl acetate (65:35), yield 26%, white solid, mp 210–213 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 2.00 (3H, s, 2-CH_3), 2.12 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.23 (1H, s, 4-CH), 7.08 (1H, s, NH), 7.26–7.46 (9H, m, aromatics), 7.55 (1H, d, J = 8.0 Hz, aromatic), 7.62 (1H, d, J = 7.4 Hz, aromatic), 7.89 (2H, d, J = 8.2 Hz, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.28, 15.20, 17.22, 39.01, 45.23, 101.69, 108.30, 111.14, 120.93, 122.96, 124.40, 125.28, 127.25, 128.26, 128.67, 129.06, 130.16, 130.30, 131.51, 135.37, 135.48, 136.60, 139.51, 148.08, 154.86, 155.26, 172.92.

4.2.26. *syn*-(±)-4-[(4-(2-Benzofuranyl)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)22]

Light petroleum-ethyl acetate (65:35), yield 25%, white solid, mp 240–242 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50 (3H, d, J = 7.2 Hz, 6-CH_3), 1.95 (3H, s, 2-CH_3), 2.10 (s, 3H, 3-CH_3), 3.92 (1H, q, J = 7.2 Hz, 6-CH), 6.72 (1H, s, 4-CH), 7.00 (1H, s, NH), 7.24–7.46 (9H, m, aromatics), 7.55 (1H, d, J = 8.0 Hz, aromatic), 7.60 (1H, d, J = 7.4 Hz, aromatic), 7.85 (2H, d, J = 8.2 Hz, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.28, 15.20, 17.212, 39.01, 45.22, 101.68, 108.29, 111.13, 120.92, 122.95, 124.39, 125.28, 127.25, 128.25, 128.66, 129.06, 130.15, 130.29, 131.51, 135.37, 135.48, 136.60, 139.50, 148.07, 154.85, 155.25, 172.92; Anal. Calcd for $C_{29}H_{25}N_3O_2S$: C, 72.63; H, 5.25; N, 8.76; O, 6.67; S, 6.69. Found: C, 72.64; H, 5.23; N, 8.77; S, 6.68.

4.2.27. *anti*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)23]

Light petroleum-ethyl acetate (80:20), yield 36%, white solid, mp 93–95 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.96 (3H, d, J = 7.2 Hz, 6-CH_3), 1.97 (3H, s, 2-CH_3), 2.14 (3H, s, 3-CH_3), 3.22 (1H, q, J = 7.2 Hz, 6-CH), 5.15 (1H, s, 4-CH), 6.95–7.50 (14H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.72, 16.34, 17.251, 36.23, 42.18, 105.04, 118.39, 119.27, 123.70, 127.51, 128.51, 129.29, 129.82, 130.58, 131.73, 135.10, 136.20, 136.38, 136.96, 147.68, 156.57, 156.86, 172.12.

4.2.28. *syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)23]

Light petroleum-ethyl acetate (80:20), yield 37%, white solid, mp 152–155 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (3H, d, J = 7.2 Hz, 6-CH_3), 1.90 (3H, s, 2-CH_3), 2.10 (3H, s, 3-CH_3), 3.81 (1H, q, J = 7.2 Hz, 6-CH), 5.63 (1H, s, 4-CH), 6.95–7.41 (14H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.34, 15.24, 17.21, 39.00, 44.91, 108.47, 118.76, 119.14, 123.56, 127.29, 128.25, 129.59, 129.78, 130.32, 131.54, 133.72, 135.30, 135.46, 136.60, 148.09, 156.71, 157.08, 172.67; Anal. Calcd for $C_{27}H_{25}N_3O_2S$: C, 71.18; H, 5.53; N, 9.22; O, 7.02; S, 7.04. Found: C, 71.38; H, 5.52; N, 9.20; S, 7.01.

4.2.29. *anti*-(±)-4-[4-(2-Methoxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)24]

Light petroleum-ethyl acetate (65:35), yield 32%, white solid, mp 201–201 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (3H, d, J = 7.2 Hz, 6-CH_3), 1.95 (3H, s, 2-CH_3), 2.20 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 3.81 (3H, s, OCH_3), 5.20 (1H, s, 4-CH), 6.90–7.50 (13H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.72, 16.30, 17.24, 36.15, 42.18, 55.85, 105.11, 112.77, 116.94, 121.10, 121.29, 125.15, 127.43, 128.50, 129.08, 130.47, 131.65, 135.20, 135.67, 136.09, 136.94, 144.52, 147.72, 151.42, 157.41, 172.29.

4.2.30. *syn*-(±)-4-[4-(2-Methoxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)24]

Light petroleum-ethyl acetate (65:35), yield 31%, white solid, mp 175–177 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (3H, d, J = 7.2 Hz, 6-CH_3), 1.87 (3H, s, 2-CH_3), 2.09 (3H, s, 3-CH_3), 3.80 (4H, m, 6-CH and OCH_3), 5.61 (1H, s, 4-CH), 6.96–7.36 (13H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.28, 15.22, 17.18, 38.96, 44.92, 55.84, 108.565, 112.73, 117.15, 121.07, 121.30, 125.09, 127.24, 128.25, 128.93, 129.37, 130.27, 131.48, 132.84, 135.23, 135.45, 136.59, 144.51, 148.12, 151.41, 157.70, 172.83; Anal. Calcd for $C_{28}H_{27}N_3O_3S$: C, 69.25; H, 5.60; N, 8.65; O, 9.88; S, 6.60. Found: C, 69.19; H, 5.61; N, 8.64; S, 6.61.

4.2.31. *anti*-(±)-4-[4-(3-Methoxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)25]

Light petroleum-ethyl acetate (65:35), yield 29%, white solid, mp 151–153 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.49 (3H, d, J = 7.2 Hz, 6-CH_3), 1.95 (3H, s, 2-CH_3), 2.15 (3H, s, 3-CH_3), 3.78 (4H, m, 6-CH and OCH_3), 5.21 (s, 1H, 4-CH), 6.5–7.5 (m, 13H, aromatics and NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.77, 16.29, 17.25, 36.18, 42.18, 55.33, 105.01, 105.28, 109.11, 111.24, 118.61, 127.45, 128.52, 129.28, 130.21, 130.50, 131.67, 135.22, 136.12, 136.64, 136.95, 147.73, 156.53, 157.82, 160.93, 172.31.

4.2.32. *syn*-(±)-4-[4-(3-Methoxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)25]

Light petroleum-ethyl acetate (80:20), yield 32%, white solid, mp 91–94 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.27 (d, J = 7.2 Hz, 3H, 6-CH_3), 1.98 (s, 3H, 2-CH_3), 2.21 (s, 3H, 3-CH_3), 3.52 (q, J = 7.2 Hz, 1H, 6-CH), 3.81 (s, 3H, OCH_3), 5.21 (s, 1H, 4-CH), 6.5–7.5 (m, 13H, aromatics and NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.77, 16.29, 17.25, 36.18, 42.18, 55.33, 105.01, 105.28, 109.11, 111.24, 118.61, 127.45, 128.52, 129.28, 130.21, 130.50, 131.67, 135.22, 136.12, 136.64, 136.95, 147.73, 156.53, 157.82, 160.93, 172.31; Anal. Calcd for $C_{28}H_{27}N_3O_3S$: C, 69.25; H, 5.60; N, 8.65; O, 9.88; S, 6.60. Found: C, 69.21; H, 5.59; N, 8.63; S, 6.61.

4.2.33. *anti*-(±)-4-[4-(4-Methoxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)26]

Light petroleum-ethyl acetate (65:35), yield 32%, white solid, mp 179–181 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (3H, d, J = 7.2 Hz, 6-CH_3), 1.90 (3H, s, 2-CH_3), 2.16 (3H, s, 3-CH_3), 3.24 (1H, q, J = 7.2 Hz, 6-CH), 3.77 (3H, s, OCH_3), 5.15 (1H, s, 4-CH), 6.91–7.47 (13H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.71, 16.31, 17.24, 36.16, 42.15, 55.60, 105.10, 114.86, 117.12, 127.44, 128.51, 129.16, 130.50, 131.65, 135.16, 135.59, 136.09, 136.94, 147.71, 149.43, 156.11, 158.03, 172.35.

4.2.34. *syn*-(±)-4-[4-(4-Methoxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)26]

Light petroleum-ethyl acetate (65:35), yield 34%, white solid, mp 97–99 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.49 (3H, d, J = 7.2 Hz, 6-CH_3), 1.83 (3H, s, 2-CH_3), 2.16 (3H, s, 3-CH_3), 3.78 (4H, m, 6-CH and OCH_3), 5.59 (1H, s, 4-CH), 6.91–7.02 (7H, m, aromatics and NH), 7.50–7.73 (6H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.29, 15.19, 17.19, 38.94, 44.86, 55.60, 108.56, 114.84, 117.49, 121.00, 127.22, 128.25, 129.45, 130.25, 131.47, 132.88, 135.25, 135.46, 136.59, 148.08, 149.58, 156.03, 158.28, 172.94; Anal. Calcd for $C_{28}H_{27}N_3O_3S$: C, 69.25; H, 5.60; N, 8.65; O, 9.88; S, 6.60. Found: C, 69.25; H, 5.59; N, 8.64; S, 6.61.

4.2.35. *anti*-(±)-4-[4-(Benzyloxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)33]

Light petroleum-ethyl acetate (75:25), yield 31%, white solid, mp 158–160 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 1.95 (3H, s, 2-CH_3), 2.20 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.10 (2H, s, OCH_2), 5.14 (1H, s, 4-CH), 6.99 (2H, d, J = 8.2 Hz, aromatics), 7.20 (2H, d, J = 8.10 Hz, aromatics), 7.30 (1H, s, NH), 7.36–7.48 (8H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.69, 16.30, 17.26, 36.13, 42.20, 70.05, 105.23, 114.78, 127.39, 127.52, 128.03, 128.56, 129.08, 130.41,

131.62, 134.19, 135.26, 136.10, 136.67, 136.95, 147.76, 158.09, 172.47.

4.2.36. *syn*-(±)-4-[4-(Benzyloxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)33]

Light petroleum-ethyl acetate (75:25), yield 32%, white solid, mp 199–203 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (3H, d, J = 7.2 Hz, 6-CH_3), 1.81 (3H, s, 2-CH_3), 2.14 (3H, s, 3-CH_3), 3.76 (1H, q, J = 7.2 Hz, 6-CH), 5.10 (2H, s, OCH_2), 5.62 (1H, s, 4-CH), 6.96 (2H, J = 8.2 Hz, aromatics), 7.20 (1H, s, NH), 7.26–7.5 (10H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.30, 15.20, 17.20, 38.91, 44.91, 70.00, 108.61, 114.98, 127.17, 127.46, 127.98, 128.25, 128.54, 129.35, 130.16, 131.43, 135.21, 135.59, 136.58, 136.70, 148.11, 158.39, 172.92; Anal. Calcd for $C_{28}H_{27}N_3O_2S$: C, 71.61; H, 5.80; N, 8.95; O, 6.81; S, 6.83. Found: C, 71.60; H, 5.79; N, 8.93; S, 6.81.

4.2.37. *anti*-(±)-4-(3-Methoxy-4-phenoxyphenyl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)34]

Light petroleum-ethyl acetate (80:20), yield 32%, white solid, mp 179–181 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 1.95 (3H, s, 2-CH_3), 2.11 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 3.80 (3H, s, OCH_3), 5.20 (1H, s, 4-CH), 6.70 (1H, d, J = 8.3 Hz, aromatic), 6.80 (1H, d, J = 8.3 Hz, aromatic), 6.90 (2H, d, J = 8.6 Hz, aromatics), 7.05 (1H, s, NH), 7.10 (1H, d, J = 8.3 Hz, aromatic), 7.30–7.46 (7H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.63, 16.21, 17.05, 36.25, 42.45, 55.86, 104.85, 112.39, 117.48, 119.61, 120.23, 122.76, 127.28, 128.34, 129.44, 130.27, 131.48, 135.22, 136.05, 136.69, 138.18, 144.73, 147.59, 151.13, 157.20, 171.98.

4.2.38. *syn*-(±)-4-(3-Methoxy-4-phenoxyphenyl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)34]

Light petroleum-ethyl acetate (80:20), yield 34%, white solid, mp 106–109 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (3H, d, J = 7.2 Hz, 6-CH_3), 1.97 (3H, s, 2-CH_3), 2.11 (3H, s, 3-CH_3), 3.75 (4H, m, 6-CH and OCH_3), 5.70 (1H, s, 4-CH), 6.80 (1H, s, NH), 6.80–7.10 (6H, m, aromatics and NH), 7.25–7.46 (7H, m, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.34, 15.21, 17.15, 38.96, 45.24, 56.03, 108.52, 112.59, 117.50, 120.58, 120.85, 122.79, 127.24, 128.27, 129.57, 130.25, 131.49, 135.41, 135.62, 135.78, 136.58, 144.98, 148.12, 151.39, 157.50, 172.87; Anal. Calcd for $C_{28}H_{27}N_3O_3S$: C, 69.25; H, 5.60; N, 8.65; O, 9.88; S, 6.60. Found: C, 69.26; H, 5.62; N, 8.64; S, 6.59.

4.2.39. *anti*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(3-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)35]

Light petroleum-ethyl acetate (75:25), yield 37%, white solid, mp 212–215 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (3H, d, J = 7.2 Hz, 6-CH_3), 2.00 (3H, s, 3-CH_3), 2.50 (s, 3H, 3-CH_3), 3.26 (1H, q, J = 7.2 Hz, 6-CH), 5.15 (1H, s, 4-CH), 7.00–7.50 (14H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.85, 16.33, 21.46, 36.44, 42.56, 106.52, 119.17, 119.91, 123.47, 124.40, 127.31, 130.15, 130.62, 136.13, 137.37, 137.74, 141.21, 148.81, 157.62, 157.75, 173.20.

4.2.40. *syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(3-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)35]

Light petroleum-ethyl acetate (75:25), yield 36%, white solid, mp 207–210 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48 (3H, d, J = 7.2 Hz, 6-CH_3), 1.97 (3H, s, 3-CH_3), 2.46 (s, 3H, 3-CH_3), 3.80 (1H, q, J = 7.2 Hz, 6-CH), 5.63 (1H, s, 4-CH), 6.96 (2H, d , J = 8.6 Hz, aromatics), 7.03 (2H, d, J = 8.6 Hz, aromatics), 7.13 (1H, t, J = 7.4 Hz, aromatic), 7.21–7.41 (9H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.30, 15.12, 21.45, 39.07, 44.90, 110.58, 119.50, 119.82, 122.84, 124.28, 126.71, 130.31, 130.41, 130.56, 134.81, 135.02, 137.98, 141.01, 149.02, 157.96, 173.81; Anal. Calcd for $C_{27}H_{25}N_3O_2S$: C, 71.18; H, 5.53; N, 9.22; O, 7.02; S, 7.04. Found: C, 71.17; H, 5.54; N, 9.21; S, 7.04.

4.2.41. *anti*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(4-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)36]

Light petroleum-ethyl acetate (80:20), yield 29%, white solid, mp 197–199 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (3H, d, J = 7.2 Hz, 6-CH_3), 1.99 (3H, s, 3-CH_3), 2.48 (s, 4-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.20 (1H, s, 4-CH), 7.00 (2H, d, J = 8.6 Hz, aromatics), 7.06 (2H, d, J = 7.8 Hz, aromatics), 7.15 (1H, t, J = 7.4 Hz, aromatic), 7.24–7.40 (9H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.73, 16.22, 21.14, 36.18, 42.29, 105.68, 118.40, 119.14, 123.60, 125.75, 129.32, 129.78, 130.56, 134.31, 135.30, 136.50, 139.27, 147.77, 156.63, 156.74, 172.04.

4.2.42. *syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(4-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)36]

Light petroleum-ethyl acetate (80:20), yield 31%, white solid, mp 97–99 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.47 (3H, d, J = 7.2 Hz, 6-CH_3), 1.99 (3H, s, 3-CH_3), 2.48 (3H, s, 4-CH_3), 3.75 (1H, q, J = 7.2 Hz, 6-CH), 5.60 (1H, s, 4-CH), 6.95 (2H, d, J = 8.6 Hz, aromatics), 7.02 (2H, d, J = 7.8 Hz, aromatics), 7.13 (1H, t, J = 7.4 Hz, aromatic), 7.27–7.37 (8H, m, aromatics), 7.50 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.18, 14.92, 21.10, 38.74, 44.55, 109.84, 117.53, 118.73, 119.05, 120.36, 123.48, 125.06, 129.60, 129.74, 130.23, 131.89, 134.07, 134.24, 134.75, 138.63, 147.91, 156.75, 156.93, 172.92; Anal. Calcd for $C_{27}H_{25}N_3O_2S$: C, 71.18; H, 5.53; N, 9.22; O, 7.02; S, 7.04. Found: C, 71.18; H, 5.54; N, 9.21; S, 7.03.

4.2.43. *anti*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-ethylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)37]

Light petroleum-ethyl acetate (80:20), yield 22%, white solid, mp 156–158 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.16 (3H, t, J = 7.6 Hz, CH_2CH_3), 1.28 (3H, d, J = 7.2 Hz, 6-CH_3), 2.00 (3H, s, 3-CH_3), 2.50 (2H, q, J = 7.6 Hz, 2H, CH_2CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.24 (1H, s, 4-CH), 6.95 (2H, d, J = 8.6 Hz, aromatics), 7.02 (2H, d, J = 7.8 Hz, aromatics), 7.13 (1H, t, J = 7.4 Hz, aromatic), 7.25–7.51 (9H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.73, 14.23, 16.31, 23.95, 36.24, 42.20, 104.98, 118.39, 119.24, 13.67, 127.38, 128.62, 129.28, 129.81, 130.00, 130.73, 134.68, 136.22, 136.45, 147.57, 156.59, 156.82, 172.30.

4.2.44. *syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-ethylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)37]

Light petroleum-ethyl acetate (80:20), yield 20%, white solid, mp 159–163 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.12 (3H, t, J = 7.6 Hz, CH_2CH_3), 1.46 (3H, d, J = 7.2 Hz, 6-CH_3), 1.90 (3H, s, 3-CH_3), 2.41 (2H, q, J = 7.6 Hz, CH_2CH_3), 3.81 (1H, q, J = 7.2 Hz, 6-CH), 5.63 (1H, s, 4-CH), 6.95–7.50 (13H, m, aromatics), 7.75 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 10.06, 11.09, 11.99, 20.71, 35.78, 41.65, 105,24, 114.02, 115.53, 115.91, 120.34, 124.02, 125.15, 126.35, 126.55, 126.70, 126.80, 127.41, 130.43, 132.23, 139.34, 144.70, 153.45, 153.85, 169.48; Anal. Calcd for $C_{28}H_{27}N_3O_2S$: C, 71.61; H, 5.80; N, 8.95; O, 6.81; S, 6.83. Found: C, 71.62; H, 5.81; N, 8.94; S, 6.83.

4.2.45. *anti*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-*n*propylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)38]

Light petroleum-ethyl acetate (80:20), yield 18%, white solid, mp 72–73 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.98 (3H, t, J = 7.3 Hz, $CH_2CH_2CH_3$), 1.26 (3H, d, J = 7.2 Hz, 6-CH_3), 1.26 (2H, m, $CH_2CH_2CH_3$), 2.00 (3H, s, 3-CH_3), 2.40 (2H, m, $CH_2CH_2CH_3$), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.24 (1H, s, 4-CH), 6.87–7.50 (13H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.72, 14.14, 16.36, 23.29, 33.18, 36.25, 42.15, 104.94, 118.43, 119.19, 123.66, 127.51, 128.65, 129.28, 129.82, 130.59, 130.84, 134.85, 136.26, 136.47, 147.45, 156.62, 156.79, 172.00.

4.2.46. *syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-*n*propylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)38]

Light petroleum-ethyl acetate (80:20), yield 20%, white solid, mp 95–99 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (3H, t, J = 7.3 Hz, $CH_2CH_2CH_3$), 1.48 (5H, m, $CH_2CH_2CH_3$ and -6-CH_3), 1.97 (3H, s, 3-CH_3), 2.35 (2H, m, $CH_2CH_2CH_3$), 3.75 (1H, q, J = 7.2 Hz, 6-CH), 5.63 (1H, s, 4-CH), 6.95–7.05 (5H, m, aromatics), 7.20–7.50 (9H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.29, 14.10, 15.18, 23.38, 33.08, 38.92, 44.78, 108.49, 118.78, 119.07, 123.52, 127.25, 128.39, 129.56, 129.77, 130.32, 130.65, 133.95, 135.14, 135.38, 141.17, 147.85, 156.75, 156.99, 172.62; IR: υ 2962.13, 1680.66, 1588.57, 1488.78, 1375.96, 1240.00, 1165.76, 1608.85, 908.79, 869.74 cm^{-1}; Anal. Calcd for $C_{29}H_{29}N_3O_2S$: C, 72.02; H, 6.04; N, 8.69; O, 6.62; S, 6.63. Found: C, 72.03; H, 6.03; N, 8.67; S, 6.62.

4.2.47. *anti*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-*i*propylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)39]

Light petroleum-ethyl acetate (80:20), yield 18%, white solid, mp 160–161 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (6H, d, J = 6.9 Hz, $CH(CH_3)_2$), 1.27 (3H, d, J = 7.2 Hz, 6-CH_3), 1.99 (3H, s, 3-CH_3), 2.60 (1H, m, $CH(CH_3)_2$), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.25 (1H, s, 4-CH), 6.95 (2H, d, J = 8.6 Hz, aromatics), 7.02 (2H, d, J = 7.8 Hz, aromatics), 7.13 (1H, t, J = 7.4 Hz, aromatic), 7.20–7.51 (9H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.78, 16.31, 23.59, 28.30, 36.15, 42.20, 104.90, 118.38, 119.25, 123.66, 127.43, 128.61, 128.28, 129.80, 130.92, 133.96, 136.44, 147.43, 156.60, 156.81, 171.97.

4.2.48. *syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-*i*propylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)39]

Light petroleum-ethyl acetate (80:20), yield 18%, white solid, mp 105–107 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (6H, d, J = 6.9 Hz, $CH(CH_3)_2$), 1.43 (3H, d, J = 7.2 Hz, 6-CH_3), 1.92 (3H, s, 3-CH_3), 2.55 (1H, m, $CH(CH_3)_2$), 3.75 (1H, q, J = 7.2 Hz, 6-CH), 5.61 (1H, s, 4-CH), 6.95 (2H, d, J = 8.6 Hz, aromatics), 7.02 (2H, d, J = 7.8 Hz, aromatics), 7.13 (1H, t, J = 7.4 Hz, aromatic), 7.20–7.51 (9H, m, aromatics and NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.29, 15.14, 23.79, 28.07, 38.88, 44.76, 108.47, 118.74, 119.11, 123.52, 126.99, 127.18, 128.39, 129.57, 129.76, 130.68, 133.95, 135.61, 147.77, 156.74, 157.01, 172.60; Anal. Calcd for $C_{29}H_{29}N_3O_2S$: C, 72.02; H, 6.04; N, 8.69; O, 6.62; S, 6.63. Found: C, 72.04; H, 6.05; N, 8.70; S, 6.62.

4.2.49. *anti*+*syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-trifluoromethylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one (40)

Light petroleum-ethyl acetate (67:33), yield 54%, white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3, A), 1.35 (3H, d, J = 7.2, 6-CH_3, B), 1.95 (3H, s, 3-CH_3, A and B), 3.25 (1H, q, J = 7.2 Hz, 6-CH, A), 3.75 (1H, q, J = 7.2 Hz, 6-CH, B), 5.22 (1H, s, 4-CH, A), 5.60 (1H, s, 4-CH, B), 7.00–7.90 (14H, m, aromatics A and B); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.78, 13.11, 14.89, 16.10, 38.55, 42.09, 44.31, 105.79, 109.50, 117.48, 118.41, 118.70, 119.12, 119.26, 120.36, 121.33, 123.51, 123.66, 124.00, 124.88, 127.70, 127.96, 129.10, 129.498, 129.73, 130.07, 130.62, 130.87, 131.06, 131.23, 131.88, 133.18, 133.44, 133.71, 134.29, 134.52, 136.22, 136.46, 137.01, 148.39, 148.57, 156.53, 156.66, 156.84, 157.02, 172.58, 173.37.

4.2.50. *anti*+*syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-fluorophenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one (41)

Light petroleum-ethyl acetate (75:25), yield 48%, white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (3H, d, J = 7.2 Hz, 6-CH_3, A), 1.40 (3H, d, J = 7.2, 6-CH_3, B), 1.95 (3H, s, 3-CH_3, A), 1.99 (3H, s, 3-CH_3, B), 3.25 (1H, q, J = 7.2 Hz, 6-CH, A), 3.77 (1H, q, J = 7.2 Hz, 6-CH, B), 5.15 (1H, s, 4-CH, A), 5.60 (1H, s, 4-CH, B), 6.90–7.60 (28H, m, aromatics A and B); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.89, 13.12, 14.77, 16.01, 36.09, 38.49, 42.16, 44.15, 106.09, 110.23, 116.75, 116.95, 117.23, 118.43, 118.72, 119.06, 119.16, 123.49, 123.62, 124.87, 125.23, 129.09, 129.22, 129.53, 129.73, 130.01, 130.84, 130.92, 131.42, 133.94, 135.80, 136.44, 148.97, 149.20, 155.14, 156.69, 156. 79, 156.95, 157.63, 172.66, 173.48.

4.2.51. *anti*+*syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-chlorophenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one (42)

Light petroleum-ethyl acetate (70:30), yield 56%, white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.27 (3H, d, J = 7.2 Hz, 6-CH_3, A), 1.35 (3H, d, J = 7.2 Hz, 6-CH_3, A), 1.95 (6H, m, 3-CH_3, A+B), 3.18 (1H, q, J = 7.2 Hz, 6-CH, A), 3.75 (1H, q, J = 7.2 Hz, 6-CH, B), 5.20 (1H, s, 4-CH, A), 5.60 (1H, s, 4-CH, B), 6.80–7.60 (28H, m, aromatics and NH, A+B); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.22, 14.94, 16.25, 35.99, 38.64, 42.12, 44.34, 105.76, 109.64, 118.43, 118.75, 119.13, 119.26, 123.54, 123.69, 128.19, 129.33, 129.24, 129.57, 129.79, 130.28, 130.58, 130.83, 131,28, 131.58, 132.35, 133.83, 134.69, 135.90, 136.51, 148.71, 148.99, 157.01, 172.60, 172.82.

4.2.52. *anti*+*syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-bromophenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one (43)

Light petroleum-ethyl acetate (65:35), yield 58%, pale yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.23 (3H, d, J = 7.2 Hz, 6-CH_3, A), 1.29 (3H, d, J = 7.2 Hz, 6-CH_3, B), 1.95 (6H, m, 3-CH_3, A+B), 3.25 (1H, q, J = 7.2 Hz, 6-CH, A), 3.82 (1H, q, J = 7.2 Hz, 6-CH, B), 5.15 (1H, s, 4-CH, A), 5.60 (1H, s, 4-CH, B), 6.95–7.75 (28H, m, aromatics and NH, A+B); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.17, 14.90, 16.25, 35.89, 38.74, 42.10, 44.34, 104.76, 108.64, 117.43, 118.75, 119.23, 119.26, 123.54, 123.69, 128.19, 129.33, 129.24, 129.57, 129.79, 130.28, 130.68, 130.83, 131,28, 131.58, 132.35, 133.78, 134.69, 135.90, 136.51, 148.59, 148.99, 157.01, 172.50, 172.80.

4.2.53. *anti*+*syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-iodophenyl)-4,8-dihydro-1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-one (44)

Light petroleum-ethyl acetate (75:25), yield 61%, pale yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (3H, d, J = 7.2 Hz, 6-CH_3, A), 1.47 (3H, d, J = 7.2 Hz, 6-CH_3, B), 1.99 (6H, s, 3-CH_3, A+B), 3.25 (1H, q, J = 7.2 Hz, 6-CH, A), 3.82 (1H, m, 6-CH, B), 5.20 (1H, s, 4-CH, A), 5.63 (1H, s, 4-CH, B), 6.80–7.55 (27H, m, aromatics and NH, A+B), 7.95 (1H, m, aromatic); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.22, 14.94, 16.26, 36.05, 38.65, 42.12, 44.34, 106.76, 110.66, 118.43, 118.75, 119.13, 120.27, 123.54, 123.69, 128.19, 129.33, 129.24, 129.72, 129.79, 130.18, 130.77, 130.83, 131,28, 131.58, 132.35, 133.93, 134.69, 135.90, 136.51, 148.71, 148.99, 157.07, 172.54, 172.78.

4.2.54. *anti*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-methoxyphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)45]

Light petroleum-ethyl acetate (67:33), yield 25%, white solid, mp 179–181 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J = 7.2 Hz, 6-CH_3), 1.95 (3H, s, 3-CH_3), 3.26 (1H, q, J = 7.2 Hz, 6-CH), 3.91 (3H, s, OCH_3), 5.20 (1H, s, 4-CH), 6.95–7.50 (13H, m, aromatics), 7.52 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.02, 16.03, 36.12, 42.32, 56.25, 105.78, 112.47, 118.47, 119.13, 121.71, 123.59, 125.91, 129.26, 129.57, 129.79, 131.02, 136.81, 148.39, 153.76, 156.70, 172.72.

4.2.55. *syn*-(±)-4-(4-Phenoxyphenyl)-3,6-dimethyl-1-(2-methoxyphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)45]

Light petroleum-ethyl acetate (67:33), yield 26%, white solid, mp 195–197 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (3H, d, J = 7.2 Hz, 6-CH_3), 2.01 (3H, s, 3-CH_3), 3.81 (1H, q, J = 7.2 Hz, 6-CH), 3.91 (3H, s, OCH_3), 5.60 (1H, s, 4-CH), 6.91–7.20 (7H, m, aromatics), 7.52–7.65 (6H, m, aromatics), 7.54 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.07, 14.80, 38.48, 44.03, 56.49, 109.77, 112.47, 118.787, 119.03, 121.88, 123.45, 126.55, 128.79, 129.51, 129.75, 130.43, 134.43, 136.10, 148.61, 152.68, 156.84, 172.32; Anal. Calcd for $C_{27}H_{25}N_3O_3S$: C, 68.77; H, 5.34; N, 8.91; O, 10.18; S, 6.80. Found: C, 68.75; H, 5.34; N, 8.90; S, 6.79.

4.3. General procedure for the synthesis of 1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-ones 15, 16, 30–32

0.1 N aqueous sodium hydroxide (50 mL) was added to a solution of the appropriate 1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-one methyl ester in THF (15 mL). The resulting suspension was stirred at room temperature for 7–8 hours. THF was removed in vacuo and the resulting aqueous phase was acidified to pH 5 and extracted with ethyl acetate (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried and the solvent removed in vacuo. The amorphous white solid thus obtained was dried at 90 °C for 12 hours.

4.3.1. *anti*-(±)-4-(3′-Carboxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)15]

Yield 95%; white solid, mp 224–227 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (3H, d, J = 7.2 Hz, 6-CH_3), 1.96 (3H, s, 2-CH_3), 2.13 (3H, s, 3-CH_3), 3.26 (1H, q, J = 7.2 Hz, 6-CH), 5.25 (1H, s, 4-CH), 7.25–7.50 (7H, m, aromatics and NH), 7.53 (1H, t, J = 7.7 Hz, aromatic), 7.60 (2H, d, J = 8.4 Hz, aromatics), 7.75 (1H, m, aromatic), 8.08 (1H, m, aromatic), 8.31 (1H, m, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.54, 16.19, 17.16, 36.16, 42.27, 104.76, 127.22, 127.39, 128.36, 128.57, 128.91, 129.80, 130.52, 131.61, 131.93, 134.80, 136.26, 139.05, 140.51, 141.42, 147.60, 170.52, 172.18.

4.3.2. *syn*-(±)-4-(3′-Carboxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)15]

Yield 96%; white solid, mp 245–248 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50 (3H, d, J = 7.2 Hz, 6-CH_3), 1.96 (3H, s, 2-CH_3), 2.13 (3H, s, 3-CH_3), 3.80 (1H, q, J = 7.2 Hz, 6-CH), 5.75 (1H, s, 4-CH), 7.25–7.50 (6H, m, aromatics), 7.52 (1H, t, J = 7.7 Hz, aromatic), 7.60 (2H, d, J = 8.4 Hz, aromatics), 7.70 (1H, s, NH), 7.75 (1H, m, aromatic), 8.02 (1H, m, aromatic), 8.27 (1H, m, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.03, 14.95, 17.11, 38.84, 44.77, 108.63, 127.06, 127.38, 128.17, 128.51, 128.61, 128.82, 129.85, 130.18, 131.32, 131.90, 135.20, 135.38, 136.48, 138.77, 139.46, 140.57, 147.85, 171.00, 173.48; Anal. Calcd for $C_{28}H_{25}N_3O_3S$: C, 69.54; H, 5.21; N, 8.69; O, 9.93; S, 6.63. Found: C, 69.53; H, 5.20; N, 8.68; S, 6.62.

4.3.3. *anti*-(±)-4-(4′-Carboxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)16]

Yield 95%; white solid, mp 255–258 °C; ^{1}H NMR ($CDCl_3$+CD_3OD, 400 MHz) δ 1.05 (3H, d, J = 7.2 Hz, 6-CH_3), 1.75 (3H, s, 2-CH_3), 1.98 (3H, s, 3-CH_3), 3.03 (1H, q, J = 7.2 Hz, 6-CH), 5.02 (1H, s, 4-CH), 7.14–7.30 (7H, m, aromatics and NH), 7.46 (2H, d, J = 8.3 Hz, aromatics), 7.52 (2H, d, J = 8.3 Hz, aromatics), 7.96 (2H, d, J = 8.3 Hz, aromatics); ^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) δ 12.78, 16.32, 17.30, 36.30, 42.47, 104.83, 126.96, 127.51, 128.42, 128.53, 130.53, 130.73, 131.68, 135.23, 136.30, 136.99, 139.05, 142.23, 145.33, 147.83, 170.93, 173.53.

4.3.4. *syn*-(±)-4-(4′-Carboxybiphenyl-4-yl)-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)16]

Yield 96%; white solid, mp 168–171 °C; ^{1}H NMR ($CDCl_3$+CD_3OD, 400 MHz) δ 1.40 (3H, d, J = 7.1 Hz, 6-CH_3), 1.88 (3H, s, 2-CH_3), 2.05 (3H, s, 3-CH_3), 3.82 (1H, q, J = 7.2 Hz, 6-CH), 5.63 (s, 1H, 4-CH), 7.27–7.40 (7H, m, aromatics and NH), 7.55 (2H, d, J = 8.3 Hz, aromatics), 7.60 (2H, d, J = 8.3 Hz, aromatics), 8.06 (2H, d, J = 8.3 Hz, aromatic); ^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) δ 12.91, 14.88, 17.03, 38.78, 44.67, 108.74, 126.78, 127.10, 127.55, 128.16, 128.60, 129.15, 130.29, 131.34, 135.35, 135.59, 136.49, 139.24, 139.58, 144.73, 148.03, 168.57, 173.20; Anal. Calcd for $C_{28}H_{25}N_3O_3S$: C, 69.54; H, 5.21; N, 8.69; O, 9.93; S, 6.63. Found: C, 69.55; H, 5.22; N, 8.68; S, 6.62.

4.3.5. *anti*-(±)-4-[4-(2-Carboxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)30]

Yield 97%; white solid, mp 135–137 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 (3H, d, J = 7.2 Hz, 6-CH_3), 1.97 (3H, s, 2-CH_3), 2.15 (3H, s, 3-CH_3), 3.21 (1H, q, J = 7.2 Hz, 6-CH), 5.20 (1H, s, 4-CH), 6.90–7.55 (13H, m, aromatics, COOH and NH), 8.21 (1H, m, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.80, 16.35, 17.28, 36.27, 42.11, 104.68, 118.49, 119.69, 124.06, 127.53, 128.50, 129.72, 130.63, 131.75, 133.47, 134.80, 136.21, 147.65, 154.52, 156.64, 166.13, 172.05;

4.3.6. *syn*-(±)-4-[4-(2-Carboxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)30]

Yield 95%; white solid, mp 127–130 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.35 (3H, d, J = 7.2 Hz, 6-CH_3), 1.80 (3H, s, 2-CH_3), 2.01 (3H, s, 3-CH_3), 3.75 (1H, q, J = 7.2 Hz, 6-CH), 5.56 (1H, s, 4-CH), 6.80–7.50 (11H, m, aromatics), 7.70 (1H, s, NH), 7.95 (1H, m, aromatic), 8.9 (1H, bs, COOH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.22, 15.10, 17.21, 38.88, 44.62, 108.59, 118.30, 119.04, 119.41, 121.06, 123.72, 127.13, 128.27, 129.47, 129.82, 130.21, 131.40, 133.00, 134.51, 135.12, 135.32, 155.70, 156.65, 167.82, 173.55; Anal. Calcd for $C_{28}H_{25}N_3O_4S$: C, 67.32; H, 5.04; N, 8.41; O, 12.81; S, 6.42. Found: C, 67.33; H, 5.03; N, 8.40; S, 6.41.

4.3.7. *anti*-(±)-4-[4-(3-Carboxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)31]

Yield 94%; white solid, mp 165–168 °C; ^{1}H NMR ($CDCl_3$+CD_3OD, 400 MHz) δ 1.24 (3H, d, J = 7.2 Hz, 6-CH_3), 1.90 (3H, s, 2-CH_3), 2.10 (3H, s, 3-CH_3), 3.18 (1H, q, J = 7.2 Hz, 6-CH), 5.15 (1H, s, 4-CH), 7.00 (2H, m, aromatics), 7.20–7.50 (9H, m, aromatics), 8.65 (1H, s, NH),

7.80 (1H, m, aromatic); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.37, 15.95, 16.98, 36.05, 41.97, 105.13, 118.56, 120.00, 123.55, 124.89, 127.28, 128.33, 129.25, 129.68, 130.46, 131.48, 132.23, 134.96, 136.16, 136.77, 147.76, 156.23, 156.60, 167.90, 172.62.

4.3.8. *syn*-(±)-4-[(4-(3-Carboxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methyphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)31]

Yield 94%; white solid, mp 173–175 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.35 (3H, d, J = 7.2 Hz, 6-CH_3), 1.80 (3H, s, 2′-CH_3), 2.02 (3H, s, 3-CH_3), 3.42 (1H, q, J = 7.2 Hz, 6-CH), 5.56 (1H, s, 4-CH), 6.98 (2H, m, aromatics), 7.20–7.50 (9H, m, aromatics), 7.60 (1H, m, NH), 7.70 (1H, m, aromatic), 8.23 (1H, bs, COOH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.19, 15.09, 17.22, 38.86, 44.65, 108.82, 118.66, 118.99, 120.15, 124.02, 125.04, 127.02, 128.34, 129.78, 131.29, 131.59, 134.45, 135.36, 136.64, 148.03, 156.46, 156.83, 169.83, 174.26; Anal. Calcd for $C_{28}H_{25}N_3O_4S$: C, 67.32; H, 5.04; N, 8.41; O, 12.81; S, 6.42. Found: C, 67.31; H, 5.02; N, 8.41; S, 6.41.

4.3.9. *anti*-(±)-4-[4-(4-Carboxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*a*(±)32]

Yield 95%; white solid, mp 220–222 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (3H, d, J = 7.2 Hz, 6-CH_3), 1.95 (3H, s, 2-CH_3), 2.13 (3H, s, 3-CH_3), 3.25 (1H, q, J = 7.2 Hz, 6-CH), 5.25 (1H, s, 4-CH), 7.05 (2H, d, J = 8.7 Hz, aromatics), 7.09 (2H, d , J = 8.6 Hz, aromatics), 7.31–7.42 (7H, m, aromatics and NH), 8.08 (2H, d, J = 8.7 Hz, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.78, 16.28, 17.25, 36.28, 42.18, 104.91, 117.55, 119.86, 124.08, 127.41, 128.27, 128.50, 129.55, 130.48, 131.64, 132.36, 135.22, 136.19, 138.16, 147.69, 154.95, 161.80, 170.50, 172.40.

4.3.10. *syn*-(±)-4-[4-(4-Carboxyphenoxy)phenyl]-3,6-dimethyl-1-(2-methylphenyl)-4,8-dihydro-1*H*-pyrazole[3,4-*e*][1,4]thiazepin-7-one [*s*(±)32]

Yield 97%, mp 168–171 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.51 (3H, d, J = 7.2 Hz, 6-CH_3), 1.82 (3H, s, 2-CH_3), 2.21 (3H, s, 3-CH_3), 3.81 (1H, q, J = 7.2 Hz, 6-CH), 5.71 (1H, s, 4-CH), 7.02 (4H, m, aromatics), 7.30–7.40 (7H, m, aromatics), 7.96 (1H, s, NH), 8.02 (2H, d, J = 8.8 Hz, aromatics); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.21 15.05, 17.221, 38.90, 44.64, 108.70, 117.44, 120.16, 124.06, 127.10, 128.29, 129.85, 130.20, 131.37, 132.28, 135.40, 135.50, 136.58, 147.97, 155.19, 161.83, 170.43, 173.81; Anal. Calcd for $C_{28}H_{25}N_3O_4S$: C, 67.32; H, 5.04; N, 8.41; O, 12.81; S, 6.42. Found: C, 67.31; H, 5.03; N, 8.40; S, 6.43.

4.4. Molecular modelling

All the PDBs used in this work were previously submitted to the *Protein Preparation Wizard* protocol of the Schrodinger Suite.[21] The DrugLike (2 million) and the LeadLike (1 million) compounds of the ZINC 8 database[20] were prepared for the calculations removing all the duplicates and generating all the tautomers, isomers and ionization states through the *Ligprep* procedure of the Schrodinger Suite. A better computational time performance was achieved transforming the resulting dataset in a *3D Phase database* through a standard procedure that adds a set of conformers to each molecule. After the backbone superposition of the five selected complexes, the ligands were extracted to identify their chemical features and create the pharmacophoric hypothesis, using the *Manage hypothesis tool* of the Phase package (present in the Schrodinger Suite). The excluded volumes were added using the residues of the superimposed protein choosing all the side-chains providing a larger volume in the binding site with the aim to create a less restrictive pharmacophore.

To verify the ability of the hypothesis to identify correctly all the agonists used for the pharmacophore generation, multiple test runs were performed using different conditions. The best performance was achieved setting the MinSites variable of the *Phase Find Matches* screening (minimum number of sites to be matched by the screened compounds to be selected as virtual hits) to 7 and leaving all the others at default. The MinSites variable of the *Phase Find Matches* screening was set to 7 while all the other variables were leaved at default.

The structure based procedure consisted in a cross docking of all the pharmacophore virtual hits in three complexes, selected to cover different binding site conformations. This procedure led to a final ranking made by six scores for each screened compound (3 X-rays per two docking programs). The lowest score value obtained by the five known agonists used in the pharmacophore generation each x-ray docking run have been used as a cut-off to select only the screened ligands with higher ranking. The grid generations on each complex and all the docking runs were performed leaving all the variables at default values. The Tanimoto clustering was performed using the CACTVS toolkit[31] and the SUBSET[32] routine.

4.5. Biology

4.5.1. FXR AlphaScreen assay

FXR activity was assessed in a recruitment coactivator assay by using AlphaScreen technology. Anti-GST-coated Acceptor beads were used to capture the GST-fusion FXR-LBD, whereas the biotinylated-SRC-1 peptide was captured by the streptavidin Donor beads. Upon illumination at 680 nm chemical energy is transferred from Donor to Acceptor beads across the complex streptavidin-Donor/Src-1-Biotin/GSTFXR-LBD/Anti-GST-Acceptor and a signal is produced. The assay was performed in white, low-volume, 384-well Optiplates (Perkin Elmer) using a final volume of 25 μl 3containing final concentrations of 10 nM of purified GST-tagged FXR-LBD protein, 30 nM biotinylated Src-1 peptide, 20 μg/ml anti-GST-coated Acceptor beads and 10 μg/ml of streptavidin Donor bead (Perkin Elmer). The assay buffer contained 50 mM Tris (pH 7.4), 50 mM KCl, 0.1% BSA, and 1 mM DTT. The stimulation times with 1 μl of tested compound (solubilized in 100% DMSO) were fixed to 30 min. at room temperature. The concentration of DMSO in each well was maintained at a final concentration of 4%. After the addition of the detection mix (acceptor and Donor beads), the plates were incubated in the dark for 4 h at room temperature and then were read in Envision microplate analyzer (Perkin Elmer). Dose response curves were done in triplicate and Z' factor was used to validate the assays. Non linear regression curves, without constraints, were performed by using four parameter equation and GraphPad Prism Software (GraphPad Inc.), to obtain the EC_{50} values.

4.5.2. FXR target gene expression

The mRNA expression level of three FXR target genes (*Ostβ, Bsep* and *Cyp7α1*) was measured by Real-Time Polymerase Chain Reaction (Q-RTPCR). Total RNA was isolated (Aurum Total RNA Mini Kit BioRad) from HepG2 cells stimulated with increasing concentration of tested compound for 18 h. The RNA was random reverse-transcribed with Iscript cDNA Synthesis kit (BioRad) in 20 μl reaction volume. Ten ng template was used in 20 μl final volume reaction of Real-Time PCR containing 0.3 μM of each primer and 10 μl of 2X SYBR Green PCR Master MIX (Bio-Rad). All reactions were performed in triplicate and the thermal cycling conditions were: 3 min at 95 °C, followed by 45 cycles of 95 °C for 10 s, and 60 °C for 30 s in iCycler iQ5 instrument (Biorad, Hercules, CA). The mean value of the replicates for each sample was calculated and expressed as cycle threshold (CT: cycle number at which

each PCR reaction reaches a predetermined fluorescence threshold, set within the linear range of all reactions). The amount of gene expression was then calculated as the difference (ΔCT) between the CT value of the sample for the target gene and the mean CT value of that sample for the endogenous control. Relative expression was calculated as the difference (ΔΔCT) between the ΔCT values of the test sample and of the control sample (WT) for each target gene. The relative quantization value was expressed and shown as 2-ΔΔCT. All PCR primers were intron spanning designed using the software Beacon Designer on published sequence data from the NCBI database.

Supplementary data

Supplementary data (general procedures for the synthesis of pyrazoles **46a–l**, 4-aryl-substituted benzaldehydes **47b–m** and 4-aryloxy-substituted benzaldehydes **47o–t**; Characterization data for compounds **46a–l**, **47e, 47i, 47k, 47l, 47p** and **47s**. Chromatographic estimation of the sample purity of 1*H*-pyrazolo[3,4-*e*][1,4]thiazepin-7-ones **8–14, 17–29, 33–45)** associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.04.021. These data include MOL files and InChiKeys of the most important compounds described in this article.

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