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1-Heteroaryl-6-(3,4-dichlorophenyl)-3-azabicyclo[4.1.0]heptane: Further insights into a class of triple re-uptake inhibitors

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ABSTRACT

Further exploration around the recently disclosed potent triple re-uptake inhibitor 6-(3,4-dichlorophenyl)-1-[(methyloxy)methyl]-3-azabicyclo[4.1.0]heptane led to the identification of a new series of potent triple re-uptake inhibitors endowed with good developability characteristics. The insertion of a further aryl moiety into the template allowed the 'titration' of the SERT/NET/DAT ratio leading to the identification of further tools in this important area.

1. Introduction

Unipolar depression is a devastating disease characterized by low mood, low self-esteem, and loss of interest or pleasure in usually enjoyable activities. In the last decades, depressed patients were treated with a number of different drugs. Amongst others, molecules able to disturb either the uptake or the metabolism of aminergic neurotransmitters have found widespread use. Tricyclic antidepressants and monoamine oxidase (MAO) inhibitors represented the 'gold standards' in the early days, but they are regrettably associated with some side effects that may hamper their efficacy.[1] More recently, molecules able to selectively block neurotransmitter re-uptake in either serotoninergic neurons (SSRI, e.g., paroxetine, Fig. 1) or noradrenergic neurons (SNRI, e.g., reboxetine, Fig. 1) became the more advanced therapies. Furthermore, drugs blocking re-uptake at both the serotoninergic and the noradrenergic transporters (e.g., venlafaxine, Fig. 1) or at both the noradrenergic and the dopaminergic neurons (e.g., bupropion, Fig. 1), also demonstrated clinical efficacy and acceptable tolerability[1]; these structures are also named as 'dual' re-uptake inhibitors.

In the last decade, the structures of new compounds able to inhibit amine re-uptake of the 5-HT, NE and DA transporters at the same time were disclosed; they are referred to as 'triple' re-uptake Inhibitors[2] (TRUI). Some of these, such as indatraline, SEP-225289, DOV derivatives are reported in Figure 1. The theory on their efficacy comes from both preclinical and clinical studies.[1,2] The simultaneous blockade of the uptake of 5-HT, NE and DA should address the anhedonic component of unipolar depression as well as shorten the time to onset of clinical efficacy because of the additional presence of the dopaminergic component with respect to 'dual' drugs. We have recently reported[3–5] the rational design of new classes of TRUI (1–3, Fig. 1) endowed with excellent potency and in vivo activity. To achieve the desired and balanced ratio among the three transporters, the use of a pharmacophore model (Fig. 2) was critical to appropriately address these three targets collectively. Actually, as previously reported[3,5], many of the disclosed TRUI show similar levels of inhibition at the three monoamine transporters (SERT ≅ NET ≅ DAT), while the new structures are endowed with a profile such that SERT ⩾ NET > DAT. The key characteristics of this model are presented in Figure 2: a positive ionisable region (P6), three hydrophobic areas (H3, H4, H5), a region in which an aromatic ring may sit (R7) and a putative H-bond acceptor zone (A2). As previously described[3,5], the presence of a secondary/tertiary amine in the template is fundamental for achieving the primary activity at the three transporters, while the decoration of the aromatic region (hydrophobic areas) provides an appropriate 'titration' of the SERT/NET/DAT components.

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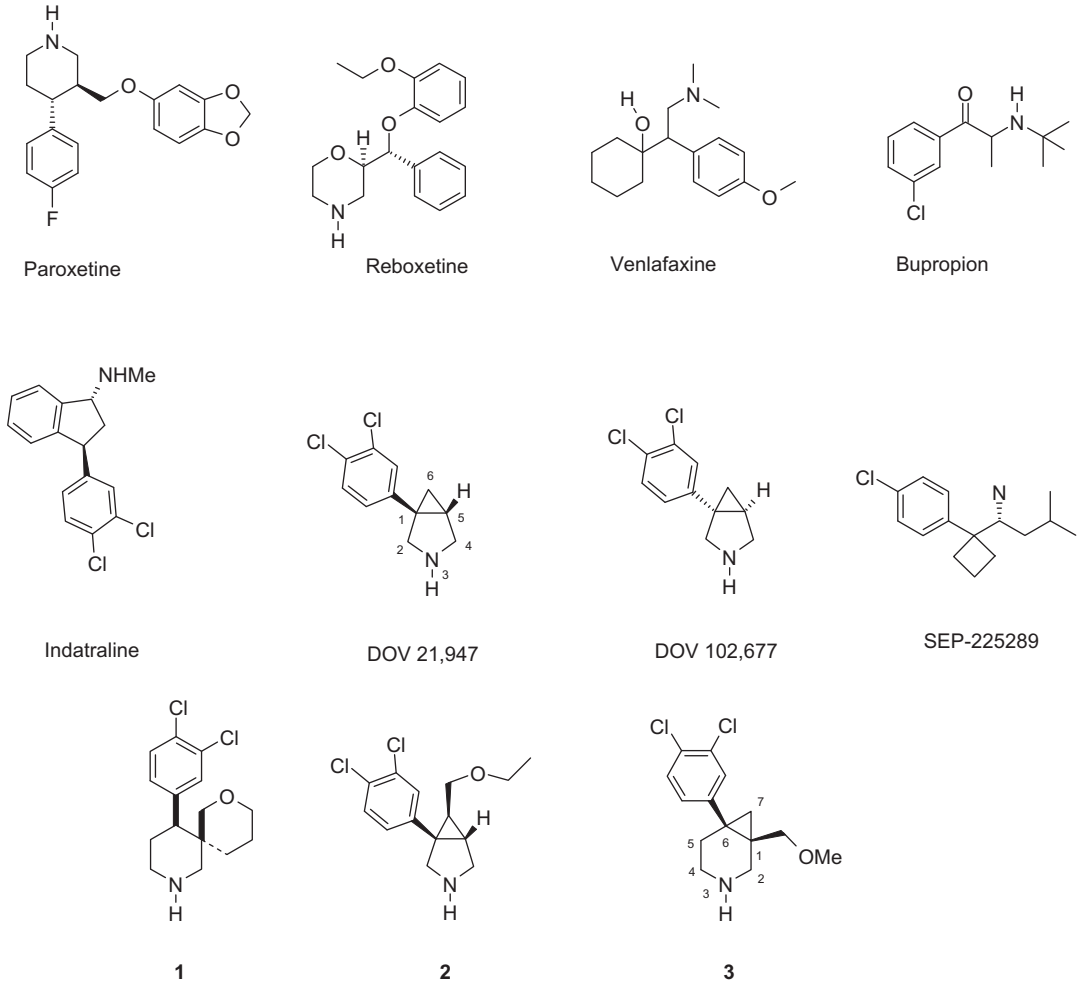


Figure 1. Structures of known monoaminergic re-uptake inhibitors.

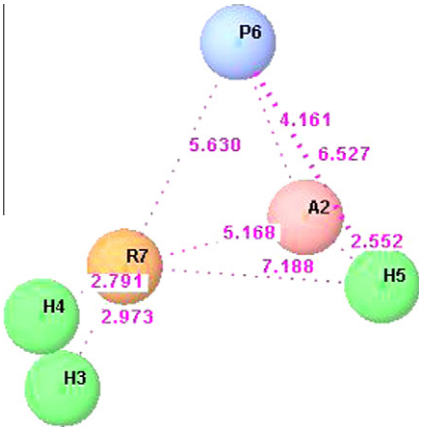


Figure 2. The triple re-uptake inhibitors pharmacophore. Coding of features: blue sphere: positive ionisable; pink spheres: H-bond acceptor; green spheres: hydrophobic; orange sphere: aromatic ring. Distances among the pharmacophoric points are shown in Ångstroms.

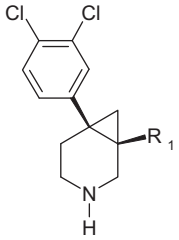
2. Results and discussion

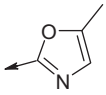
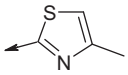
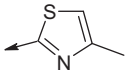
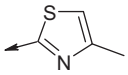
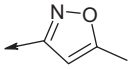
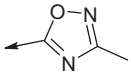
From the previous exploration (Table 3, Ref. 5) it resulted quite clear that the region of space represented by the feature A2-H5 of the pharmacophore model was fairly tolerant to the presence of hindered substituents. Additionally, the same region also proved to be relatively tolerant to the presence of sp^2-hybridized systems such as ketones or amides (Table 4, Ref. 5). For these reasons, the decision to explore this area with C-1 linked heterocycles was taken. The synthetic paths utilized to prepare the compounds reported in Tables 1–7 are outlined in Scheme 1. Starting from the versatile intermediate 44[5,6], it was possible to prepare both C-linked and N-linked heterocycles.

The potential topological mapping of the methoxy group (derivative 3[5], Table 1) within a five-membered heterocycle led to the synthesis of compound **4** (Table 1). Despite higher MW and volume (MW = 301 vs 280 Da; polar surface area (PSA) = 38 vs 21 Å,[2] respectively), the racemic derivative **4** showed similar affinity values with respect to compound **3**. The main variation was represented by the DAT affinity, which was higher than the other two components.

Accordingly, the ratio among the transporter affinities moved from a SERT/NET/DAT ≅ 1/10/10 proportion to a 10/10/1 profile. This specific feature was of great interest as this discovery provided a new tool with a different profile with respect to those previously described. It is important to notice, however, that in such a derivative both the oxygen and the nitrogen may have played the role of the H-bond acceptor mapped by the pharmacophore model, potentially leading to such a difference. To further investigate this point, a further derivative was designed. The oxygen atom of derivative **4** was replaced with a sulphur atom, a worse H-bond acceptor, leading to the synthesis of product **5** (Table 1).

Table 1
Binding at the three transporters (SERT, NET, DAT) expressed as pK_i



Entry	Stereo-chemistry	R_1	hSERT SPA pK_i	hNET SPA pK_i	hDAT SPA pK_i	*c* log P*	TPSA**
DOV 21,947	(s.e.)	N.A.	7.8	7.2	7.10	3.5	12
Indatraline	(s.e.)	N.A.	9.7	8.8	9.1	4.6	12
1	(s.e.)	N.A.	9.5	8.4	7.9	3.6	21
2	(s.e.)	N.A.	9.8	9.3	8.1	4.1	21
3	(s.e.)	CH_2OMe	9.2	8.1	8.0	3.1	21
4	(rac.)		8.2	8.2	9.0	2.7	38
5	(rac.)		9.3	9.4	9.8	3.8	25
6	(s.e.)		9.4	9.4	9.7	3.8	25
7	(s.e.)		7.8	7.5	7.7	3.8	25
8	(rac.)		8.1	7.4	8.7	2.3	43
9	(rac.)		8.1	7.5	9.1	2.4	51

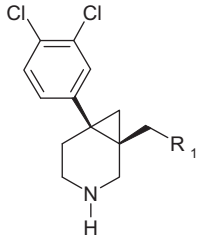
Only relative stereochemistry is shown; SEM for hSERT/NET/DAT data sets is ± 0.1;(rac.) = racemate; (s.e.) = single enantiomer; N.A. = Not applicable.
* ACD-*c* log *P*.
** ACD TPSA.

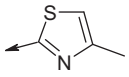
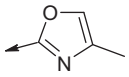
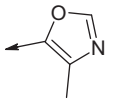
The new derivative showed a higher *c* log *P*[7] (3.8 vs 2.7) with respect to compound **4**, while its PSA was more similar to derivative **3** (25 vs 21). The resulting SERT/NET/DAT ratio for this compound was intermediate at ≅3/3/1. To verify if, as in the previously reported series, the affinity was mainly present in one of the enantiomers, the racemic mixture was separated by chiral HPLC. The results for derivatives **6** and **7** are reported in Table 1. Also in this series, at least 40- to 50-fold separation of affinity was present between the eutomer and the distomer. Accordingly, to simplify the exploration of the new series, all compounds were prepared as racemates and only the most interesting derivatives were submitted to chiral separation, unless synthetic problems prevented proceeding in such a way. Considering the sub-nanomolar affinity achieved for each transporter, derivative **6** was further characterized in agreement with the screening cascade previously described.[3–5] The IC_{50} values for all major P450 isoforms tested (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) were greater than 6 μM with the exception of CYP2D6 (4 μM) and intrinsic clearance (CLi) values, both in human and in rat, were moderate to high (2.6 and 5.4 ml/min/g of protein). Further investigations were performed with both the isoxazole and the oxadiazole derivatives (compound **8** and **9**, respectively) to verify if the α-effect of the two neighbouring heteroatoms might have played a role on the affinity values for the three transporters. Comparing the results achieved for these two products (Table 1) with the oxazole derivative **4**, it is possible to notice a slight variation on NET affinity only; the other values remained almost unchanged.

Considering the change in the SERT/NET/DAT profile and, according to the pharmacophore model predictions, the potential availability of further space around the heterocyclic moiety it was decided to expand the investigation of the role of the heterocycles, adding a methylene group between these groups and the [4.1.0] scaffold. The compounds were prepared in accordance to Scheme 1. The homologated thiazole 10 (Table 2) showed subnanomolar affinity at SERT and a SERT/NET/DAT ratio ≅ 1/10/100. The regioisomeric oxazoles (**11**, **12**) showed lower affinity at the three transporters with a similar 1/10/10 ratio. On the basis of these results, considering the complex chemical synthesis of these C-linked derivatives and given the availability of the intermediate **44** (Scheme 1), the decision to explore also N-linked heterocycles was taken. In this case, when dealing with heterocycles in which more than one nitrogen atom was present in the ring, the possibility to have regioisomeric derivatives was also considered. Results achieved with the exploration of the pyrazole system are reported in Table 3.

The exploration started with commercially available 3(5)-methyl pyrazole, where the nitrogen α to the N-linker should have played the role of H-bond acceptor. As expected, two regioisomers were obtained from the S_N2 reaction on the intermediate mesyl derivative (Scheme 1) leading to the racemic mixture **13**. This mix-

Table 2
Binding at the three transporters (SERT, NET, DAT) expressed as pK_i



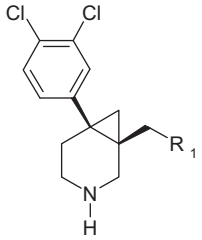
Entry	Stereo-chemistry	R_1	hSERT SPA pK_i	hNET SPA pK_i	hDAT SPA pK_i
10	(rac.)		10.0	9.2	8.2
11	(rac.)		8.7	7.5	7.2
12	(rac.)		8.5	7.6	7.9

Only relative stereochemistry is shown; SEM for hSERT/NET/DAT data sets is ± 0.1;(rac.) = racemate.

ture was tested as such to shed light on this initial N-linked derivative. Considering the nanomolar affinity at both SERT and NET and the good potency at DAT (SERT/NET/DAT ratio ≅ 1/5/20), it was decided to examine the single components of this mixture. The analytical separation of the regioisomers was only possible on chiral columns leading directly to the evaluation of the four single enantiomers reported in Table 3 (**14**–**17**). In keeping with the findings for thiazole **5**, for each regioisomer (**14**, 15 vs **16**, **17**) one of the two enantiomers showed a better affinity profile at the three transporters. Interestingly enough, the positioning of a single methyl group (**15** vs **17**) determined almost no difference at NET, but proved to have a somewhat greater, but opposing, effect at SERT and DAT; in derivative **15**, the 3-methyl group on the pyrazole led to a higher SERT activity and a lower DAT activity than derivative **17** with the 5-methyl group. The IC_{50} values for all major P450 isoforms tested for compound 15 were greater than 10 μM with the exception of CYP2D6 (2 μM).

Compound **17** showed a superimposable P450 profile, and it was also tested for intrinsic clearance; values both in human and in rat resulted moderate (2.5 and 2.6 ml/min/g of protein, respectively). To achieve a potentially 'intermediate' situation, derivative **18** was planned considering that only one regioisomer was possible with such a substitution pattern. Quite unexpectedly, the affinity at both NET and DAT showed a drastic reduction leading to a SERT/NET/DAT ratio ≅ 1/100/100. Accordingly, it might be hypothesized, considering the electron neutrality of the group, that the presence of a methyl group in position 4 of the pyrazole ring might negatively affect the binding at both NE and DA transporters because of steric hindrance. The next logical step was therefore to verify the effect of two methyl groups in positions 3 and 5 of the pyrazole moiety. Compound **19** was therefore synthesized, leading to an interesting SERT/NET/DAT ratio ≅ 1/4/16. Given the high affinity at SERT, the racemate was separated into its two enantiomers (**20**, **21**). Compound **21** showed nanomolar affinity at SERT and NET, confirming a SERT/NET/DAT ratio ≅ 1/5/16. The IC_{50} values for the P450 isoforms tested for this compound were greater than 8 μM with the exception of CYP2D6 (2 μM); CLi values in human and in rat resulted moderate to high (2.4 and 5.5 ml/min/g of protein, respectively). To fully understand the value associated to

Table 3
Binding at the three transporters (SERT, NET, DAT) expressed as pK_i



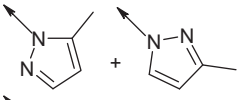
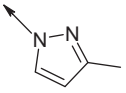
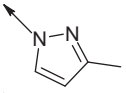
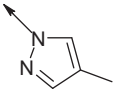
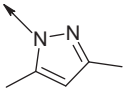
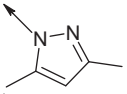
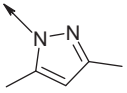
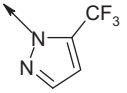
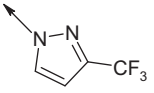
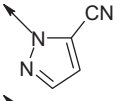
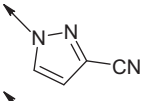
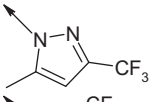
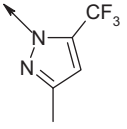
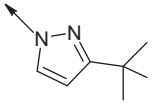
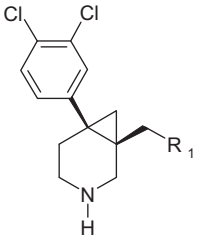
Entry	Stereo-chemistry	R_1	hSERT SPA pK_i	hNET SPA pK_i	hDAT SPA pK_i
13	(rac.)		9.8	9.1	8.5
14	(s.e.)		7.7	6.0	6.5
15	(s.e.)		9.6	8.5	7.8
16	(s.e.)		7.7	6.5	7.0
17	(s.e.)		9.1	8.8	8.5
18	(rac.)		9.1	7.1	6.9
19	(rac.)		9.4	8.8	8.2
20	(s.e.)		7.8	6.3	6.8
21	(s.e.)		9.7	9.0	8.5
22	(rac.)		8.7	8.0	8.8
23	(rac.)		8.7	6.8	7.6
24	(rac.)		8.3	7.5	7.7
25	(rac.)		9.0	7.6	7.6
26	(rac.)		9.0	7.6	8.7
27	(rac.)		9.1	8.0	8.9

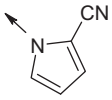
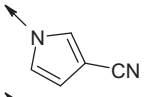
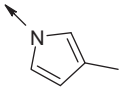
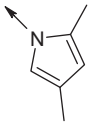
Table 3 (*continued*)

Entry	Stereo-chemistry	R_1	hSERT SPA pK_i	hNET SPA pK_i	hDAT SPA pK_i
28	(rac.)		9.6	7.3	8.2

Only relative stereochemistry is shown; SEM for hSERT/NET/DAT data sets is ± 0.1;(rac.) = racemate; (s.e.) = single enantiomer.

Table 4
Binding at the three transporters (SERT, NET, DAT) expressed as pK_i

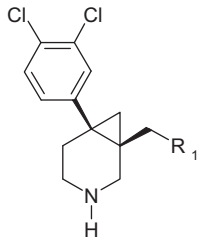


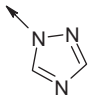
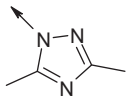
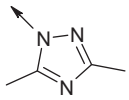
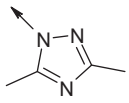
Entry	Stereo-chemistry	R_1	hSERT SPA pK_i	hNET SPA pK_i	hDAT SPA pK_i
29	(rac.)		9.4	8.0	7.8
30	(rac.)		8.6	7.4	7.9
31	(s.e.)		9.2	7.5	7.9
32	(s. e.)		9.3	7.4	7.5
33	(rac.)		8.6	7.7	7.7
34	(rac.)		8.7	7.7	8.1

Only relative stereochemistry is shown; SEM for hSERT/NET/DAT data sets is ± 0.1;(rac.) = racemate; (s.e.) = single enantiomer.

this new series, the pharmacokinetics (PK) and oral bioavailability of **21** were investigated in rat.[8] In good agreement with in vitro data, compound **21** showed low bioavailability (F = 6%), moderate distribution volume (Vd = 6.9 l/kg), and moderate blood clearance (Clb = 52 ml/min/kg) resulting in a half-life of 2.3 h. The average Brain:Blood (B/B) concentration ratio was equivalent to 0.8 at 1 h. Considering that the fraction of the compound absorbed (Fa) and the hepatic extraction (E_H) were high (Fa = 50%, E_H = 0.9), it was hypothesized that the pyrazole ring might have been prone to some metabolic degradation. Consequently, to reduce the electron rich character of the methyl pyrazole, electron-withdrawing groups were added to the pyrazole nucleus (**22**–**27**, Table 3). Such an alteration could also play a role on the H-bond acceptor capabilities of the system, modifying the affinity at the transporter. From the chemical reactivity point of view, it was interesting to notice

Table 5
Binding at the three transporters (SERT, NET, DAT) expressed as pK_i



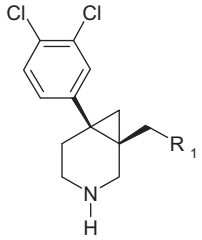
Entry	Stereo-chemistry	R_1	hSERT SPA pK_i	hNET SPA pK_i	hDAT SPA pK_i
35	(rac.)		8.4	7.0	6.7
36	(rac.)		8.5	7.4	7.6
37	(s.e.)		8.7	7.6	7.8
38	(s. e.)		7.4	5.8	6.7

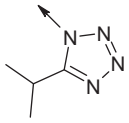
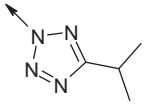
Only relative stereochemistry is shown; SEM for hSERT/NET/DAT data sets is ± 0.1;(rac.) = racemate; (s.e.) = single enantiomer.

that for these derivatives, it was possible to separate the regioisomers on achiral HPLC column and therefore without the need to isolate each single enantiomer. The introduction of a single –CF_3 group in position 5 of the pyrazole scaffold (**22**) determined a slight decrease of affinity on NET when compared with the single enantiomer **17**. On the other hand, the introduction of such a group in position 3 (**23**) determined a decrease of the affinity towards SERT and NET when compared to the single enantiomer **15**. This outcome was probably linked to the electron-withdrawing effect on the nitrogen atom α to the –CF_3 group, strongly reducing its ability as a H-bond acceptor. The greater electron-withdrawing effect of the cyano moiety showed a different behaviour on the two regioisomers. The 5-CN derivative (**24**) showed a further decrease in affinity at the three transporters when compared to both the trifluoromethyl derivative **22** and the methyl analogue **17** (as a single enantiomer). On the contrary, the 3-CN derivative (**25**) was less active on SERT and NET when compared to the single enantiomer of the methyl derivative 15, but it was more active than derivative **23** on these two transporters. Once again, NET seemed to be the most sensitive transporter to these stereoelectronic variations. Considering that the –CN group is more electron-withdrawing than the –CF_3 moiety according both to Hammett and to Swain and Lupton parameters, this latter result might appear difficult to explain. In fact, the nitrogen α to the electron-withdrawing group should be more deactivated as a H-bond acceptor when in proximity to the –CN group. Consequently, it might be hypothesized that the nitrogen of the nitrile is itself able to form an alternative H-bond with neighbouring residues or that it might favour potential edge-to-face aromatic interactions of the pyrazole itself with appropriate amino acid residues present in that region.

The replacement of a single methyl group in compound **19** with a –CF_3 group, led to a slightly reduced affinity for SERT and to a greater reduction for NET for both regioisomers (**26**, **27**); in con-

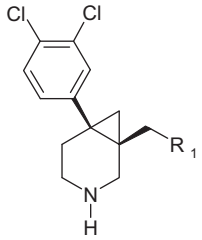
Table 6
Binding at the three transporters (SERT, NET, DAT) expressed as pK_i

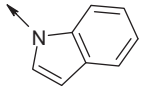
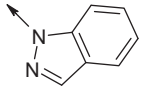
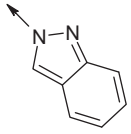


Entry	Stereo-chemistry	R_1	hSERT SPA pK_i	hNET SPA pK_i	hDAT SPA pK_i
39	(rac.)		7.2	6.4	7.2
40	(rac.)		9.1	6.4	7.6

Only relative stereochemistry is shown; SEM for hSERT/NET/DAT data sets is ± 0.1;(rac.) = racemate; (s.e.) = single enantiomer.

Table 7
Binding at the three transporters (SERT, NET, DAT) expressed as pK_i



Entry	Stereo-chemistry	R_1	hSERT SPA pK_i	hNET SPA pK_i	hDAT SPA pK_i
41	(s.e.)		8.9	7.2	7.7
42	(s.e.)		9.6	7.3	7.8
43	(s.e.)		9.7	7.3	8.4

Only relative stereochemistry is shown; SEM for hSERT/NET/DAT data sets is ± 0.1;(rac.) = racemate; (s.e.) = single enantiomer.

trast, these modifications led to increased DAT values, independently of the position (3 or 5) of the $-CF_3$ moiety on the pyrazole ring. The final compound to be prepared in this mini-series was the 3-*t*-butyl pyrazole **28**. A single regioisomer was achieved from the nucleophilic substitution of the mesyl intermediate; this is probably due to the poor accessibility of the nitrogen adjacent to the hindered *t*-butyl moiety for steric reasons. When compared to the single enantiomer 3-methyl derivative **15**, it may be noticed that the affinity at SERT and DAT are quite comparable, while the NET affinity dropped off about one logarithmic unit, confirming the sensitivity of this transporter to steric hindrance in this area.

To further investigate the role of the H-bond acceptor in the N-linked heterocyclic scaffolds, it was decided to completely remove the second nitrogen from the template using a pyrrole nucleus. The results generated on this mini-series are reported in Table 4. The unsubstituted pyrrole derivative (compound **29**) showed nanomolar potency at SERT, comparable to that achieved with the methyl pyrazole **13**, while the affinity on NET was one logarithmic unit lower and a slightly lower decrease was also observed on DAT. A direct comparison between the two systems can be made between the 2-CN pyrrole **30** and the 5-CN pyrazole **24** (also available as racemate), where the position of the substituent is equivalent. Considering that the two profiles are almost superimposable, it might be possible to conclude that the electron-withdrawing effect of the cyano group on the pyrazole system was enough to reduce to a minimum the H-bond acceptor capabilities of that template, even when the group was distal from the nitrogen. A similar, but indirect, comparison can also be made between the pyrrole **31** and the pyrazole **25**. In this case, as the pyrrole racemate was not pure enough for biological testing, the two single enantiomers were separated by chiral HPLC and only the results of the most active enantiomer are reported in Table 4.

In any event, also in this case the two profiles are superimposable and the higher SERT affinity of **31** relative to **25**, even if not statistically significant, might be related to the greater values derived from testing the single enantiomer versus the racemate. The same situation is valid for the SERT affinity in derivative **32**; also in this case a single enantiomer for the above mentioned reason.

Again for this derivative, as for the pyrazole **18**, lower affinity values were achieved on NET and DAT. The 2,5-dimethyl derivative **33**, where both the positions in which the putative H-bond acceptor might sit in the pyrazole nucleus are hindered, showed a statistically significant difference when compared to the dimethyl derivative **19**. This reduction was comparable to that observed for derivative **34**, where the two methyl groups were in exactly the same position as 19. Comparing this latter result, it may be observed that the lack of the pyrazole nitrogen seems to have a greater effect on SERT and NET, while no major difference can be noticed at DAT. It might therefore be postulated that for SERT and NET, the presence of the putative H-bond acceptor is beneficial for affinity, considering also that the pyrrole **34** showed a significant increase in *c* log *P* with respect to the pyrazole 19 (*c* log *P* = 5.2 vs 3.8, respectively). No major difference between the pyrazole and pyrrole mini-series was observed in terms of in vitro DMPK.

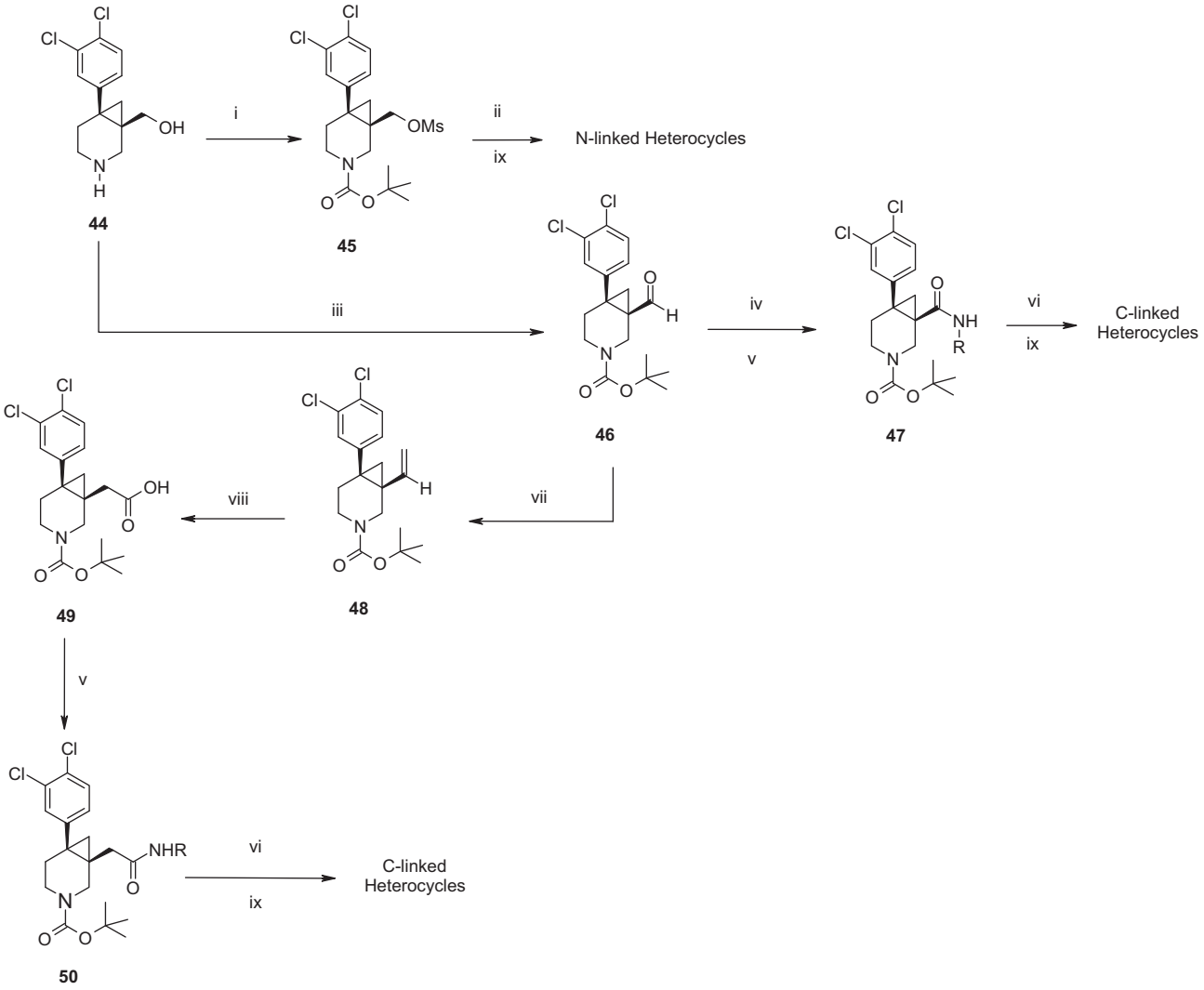
To examine the response of the transporter systems to the introduction of further potential H-bond acceptors and to a reduction in *c* log *P* of the heterocycles, further compounds were designed. Table 5 reports the results for two triazoles, while Tables 6 reports the results of two regioisomeric tetrazoles. Derivative 35 (*c* log *P* = 2.3) showed a clearly reduced lipophilicity versus the pyrrole 29 (*c* log *P* = 4.2). The affinity values at the three transporters showed a one logarithmic unit decrease. The introduction of two methyl groups to help counterbalance the drop in lipophilicity (derivative **36**, *c* log *P* = 2.8) gave no major improvement from the affinity point of view. The racemate was separated into the single enantiomers (**37**, **38**) to evaluate the effects of such a reduction in lipophilicity on the in vitro and in vivo PK profile.

The IC_{50} values for all major P450 isoforms tested for derivative **37** were greater than 25 μM. From the in vivo point of view, the compound was investigated in rat[8] showing, low to moderate bioavailability (F = 17%), moderate to high distribution volume (Vd = 8.1 l/kg), and moderate to high blood clearance (Clb = 63 ml/min/kg) resulting in a half-life of 1.7 h. The average Brain:Blood (B/B) concentration ratio was equivalent to 0.4 at 1 h. Considering that the fraction of the compound absorbed, as well as the hepatic extraction, was high (Fa = 94%, E_H = 0.8), it was confirmed that also this specific derivative suffered from high metabolism.

Two regioisomers were attained utilizing the 'symmetric' 5-isopropyl tetrazole, (39, 40. *c* log *P* = 3.7 and 3.8, respectively). Despite a comparable *c* log *P* with compound **19** (*c* **log** *P* = 3.8), it could be clearly appreciated that these derivatives generated a completely different affinity profile at the three transporters. Considering the steric hindrance of the isopropyl group of 39 on one side of the scaffold and the 'hydrophilic' region generated by the three remaining nitrogens on the other side of the template, it might be postulated that such an arrangement caused a major misalignment within the binding pockets of the transporters. For compound **40**, where the steric hindrance of the *i*-Pr group pointed into the same region of space as the methyl group of derivative **18**, the maximum detrimental effect was obtained on NET, confirming the sensitivity of this transporter to such effects, while almost no effect was observed on SERT when compared to both **18** and **19**.

Finally, to complete the exploration, benzofused systems were explored. Results for the indole **41** and for the regioisomeric indazoles (**42**, **43**) are reported in Table 7. The increased hindrance of these derivatives hampered their affinity on NET. Their increased lipophilicity (*c* log *P* = 5.6 for **41** and 4.7 for **42**, **43**) had no major impact on SERT and DAT, but once again it could be observed that the presence of a potential H-bond acceptor in the indazole system improved the SERT component with respect to the indole derivative. A potentially more favourable orientation of **43** (similar in shape to **40**) also improved the DAT component with respect to **42**.

In the end, it is possible to state that the exploration of the 3-azabicyclo[4.1.0]heptane pharmacophore model using different heteroaromatic groups allowed the identification of region of space within the three transporters able to accommodate both directly linked heterocycles (**4**–**9**) and appropriately spaced systems (**10**–**43**). Depending on the nature of the heterocycle, a different ratio among the three transporters was achieved and fine tuned (**4**–**12**). The spaced N-linked pyrazoles (**13**–**28**) showed profiles closer to compound **3** in terms of SERT/NET/DAT ratio, but this was very sensitive to the specific nature of the substituents and to their position on the ring. The use of a N-linked pyrrole system (**29**–**34**) was also well tolerated as well as the use of heteroaromatic systems with more than two nitrogens (**35**–**40**), leading to a variety of tools for the medicinal chemist. Benzofused systems (**41**–**43**),



Scheme 1. General synthetic procedures. Reagents and conditions: (i): (a) BOC_2O, TEA, CH_2Cl_2, from 0 °C to RT, 4 h; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C, 1 h; ii: HN-Heterocycle, NaH, DMF, 0 °C; (iii): Dess–Martin Periodinane, CH_2Cl_2, from 0 °C to RT, 3 h; (iv): Jones Reagent, acetone, 0 °C; (v): TBTU, DIPEA, 0 °C followed by RNH_2 or NH_2OH; (vi): (a) DMF, appropriate halocarbonyl derivative from RT to 60 °C; b) for sulfur containing heterocycles, step a) was performed after transformation of the amide into the corresponding thioamide via P_2S_5 or Lawesson's reagent, DMF, 6 h vii: $MePPh_3Br$, BuLi, THF, from 0 °C to RT, 18 h; viii: a) BH_3-THF, THF, RT, 4 h; b) H_2O_2 0 °C; c) Jones reagent, acetone, 0 °C; ix) TFA, CH_2Cl_2, 0 °C.

despite their bulkiness, also demonstrated to be well tolerated by the three transporters, with the NET one being the most sensitive to the different ring variations.

3. Conclusions

The discovery of the 1-heteroaryl-6-(3,4-dichlorophenyl)-3-azabicyclo[4.1.0]heptane template as a potent class of triple re-uptake inhibitors is reported. Detailed SAR information is reported for a number of derivatives, allowing the identification of patterns of activity related to the different heteroaromatic moieties used in the exploration. A number of new tool molecules with different SERT/NET/DAT ratios were made available for in vivo experiments. Clear trends of activity for each of the transporters were outlined. The molecules belonging to this class are endowed with good developability profiles. In vivo PK data for selective derivatives are also reported.

4. Experimental section

4.1. Biological test methods

4.1.1. In vitro studies

4.1.1.1. SPA-binding for human recombinant SERT, NET and DAT. The affinity of the compounds to the human transporters has been assessed with radioligand displacement binding of [^{3}H]citalopram, [^{3}H]nisoxetine and [^{3}H]WIN-35,428 for SERT, NET, and DAT in BacMam-recombinant human SERT, NET and DAT membranes with the SPA technology. Briefly, 0.3 μL of test compound were added by 30 μL of the SPA mixture, containing 1 mg/ml SPA (GE HealthCare, RPNQ0260) beads (SERT) or 2 mg/ml SPA beads (NET and DAT), 6 or 40 or 20 μg/ml of SERT or NET or DAT BacMam membranes, 0.02% Pluronic F-127, 3 nM [^{3}H]citalopram, or 10 nM [^{3}H]nisoxetine or 10 nM [^{3}H]WIN-35,428 for SERT or NET or DAT binding SPA in the assay buffer (20 mM HEPES, 145 mM NaCl, 5 mM KCl, pH 7.4). Incubation was performed overnight at room temperature. Bound radioactivity was measured with the Viewlux instrument. Data analysis was performed by using a 4-parameter logistic equation with ActivityBase software and pK_i has been calculated from pIC_{50} by using the Cheng–Prusoff equation.

4.1.1.2. P450 CYPEX assay. Inhibition (IC_{50}) of human CYP1A2, 2C9, 2C19, 2D6 and 3A4 was determined using Cypex Bactosomes expressing the major human P450s. A range of concentrations (0.1, 0.2, 0.4, 1, 2, 4, and 10 μM) of test compound were prepared in methanol and pre-incubated at 37 °C for 10 min in 50 mM potassium phosphate buffer (pH 7.4) containing recombinant human CYP450 microsomal protein (0.1 mg/mL; Cypex Limited, Dundee, UK) and probe-fluorescent substrate. The final concentration of solvent was between 3% and 4.5% of the final volume. Following pre-incubation, NADPH regenerating system (7.8 mg glucose 6-phosphate, 1.7 mg NADP and six units glucose 6-phosphate dehydrogenase/ml of 2% (w/v) $NaHCO_3$; 25 μL) was added to each well to start the reaction. Production of fluorescent metabolite was then measured over a 10 min time-course using a Spectrafluor plus plate reader. The rate of metabolite production (AFU/min) was determined at each concentration of compound and converted to a percentage of the mean control rate using Magellan (Tecan software). The inhibition (IC_{50}) of each compound was determined from the slope of the plot using Grafit v5 (Erithacus software, UK). Miconazole was added as a positive control to each plate. CYP450 isoform substrates used were ethoxyresorufin (ER; 1A2; 0.5 μM), 7-methoxy-4-triflouromethylcoumarin-3-acetic acid (FCA; 2C9; 50 μM), 3-butyryl-7-methoxycoumarin (BMC; 2C19; 10 μM), 4-methylaminomethyl-7-methoxycoumarin (MMC; 2D6; 10 μM), diethoxyflourescein (DEF; 3A4; 1 μM) and 7-benzyloxyquinoline (7-BQ; 3A4; 25 μM). The test was performed in three replicates.

4.1.1.3. Intrinsic clearance (CLi) assay. Intrinsic clearance (CLi) values were determined in rat and human liver microsomes. Test compounds (0.5 μM) were incubated at 37 °C for 30 min in 50 mM potassium phosphate buffer (pH 7.4) containing 0.5 mg microsomal protein/mL. The reaction was started by addition of co-factor (NADPH; 8 mg/mL). The final concentration of solvent was 1% of the final volume. At 0, 3, 6, 9, 15, and 30 min an aliquot (50 μL) was taken, quenched with acetonitrile containing an appropriate internal standard and analysed by HPLC–MS/MS. The intrinsic clearance (CLi) was determined from the first order elimination constant by non-linear regression using Grafit v5 (Erithacus software, UK), corrected for the volume of the incubation and assuming 52.5 mg microsomal protein/g of protein for all species. Values for CLi were expressed as ml/min/g of protein. The lower limit of quantification of clearance was determined to be when <15% of the compound had been metabolised by 30 min and this corresponded to a CLi value of 0.5 ml/min/g of protein. The upper limit was 50 ml/min/g of protein.

4.2. Chemical procedures

4.3.1. General

Proton Magnetic Resonance (NMR) spectra are typically recorded either on Varian instruments at 300, 400 or 500 MHz, or on a Bruker instrument at 300 and 400 MHz. Chemical shifts are reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. The NMR spectra were recorded at a temperature ranging from 25 to 90 °C. When more than one conformer was detected the chemical shifts for the most abundant one is reported. Mass spectra (MS) are typically taken on a 4 II triple quadrupole Mass Spectrometer (Micromass UK) or on a Agilent MSD 1100 Mass Spectrometer, operating in ES (+) and ES (−) ionization mode or on an Agilent LC/MSD 1100 Mass Spectrometer, operating in ES (+) and ES (−) ionization mode coupled with HPLC instrument Agilent 1100 Series. In the mass spectra only one peak in the molecular ion cluster is reported. When HPLC walk-up retention time is reported, the analysis is done on a HPLC Agilent 1100 Series Instrument with the following method: Column: Luna C18 100A 50 × 2 mm, 3 μm; Mobile Phase: (MeCN + 0.05% TFA)/(H_2O + 0.05% TFA) gradient 0/100 to 95/5 in 8 min; Flux 1 ml/min. Flash Silica gel chromatography was carried out on Silica gel 230–400 mesh (supplied by Merck AG Darmstadt, Germany) or over Varian Mega Be-Si pre-packed cartridges or over pre-packed Biotage Silica cartridges. SPE-SCX cartridges are ion exchange solid phase extraction columns supplied by Varian. The eluent used with SPE-SCX cartridges is methanol followed by 2 N ammonia solution in methanol. In a number of preparations, purification was performed using either Biotage manual flash chromatography (Flash+) or automatic flash chromatography (Horizon, SP1) systems. All these instruments work with Biotage Silica cartridges. SPE-Si cartridges are Silica solid phase extraction columns supplied by Varian. The enantiomeric purity of each single enantiomer obtained after preparative chromatography on chiral columns, was always verified on analytical column. The purity of the compounds reported in the manuscript was established through HPLC methodology. All the compounds reported in the manuscript have a purity >95%.

4.3.2. General synthetic procedures

Detailed experimental part for each compound can be also found in Ref. 9.

4.3.2.1. 1,1-Dimethylethyl-6-(3,4-dichlorophenyl)-1-{[(methylsulfonyl)oxy]methyl}-3-azabicyclo[4.1.0]heptane-3-carboxylate 45. To a stirred solution of methyl derivative **44**[5,6] in dry CH_2Cl_2, at 0 °C and under a nitrogen atmosphere, triethylamine was added dropwise followed by mesyl-Cl and the mixture was stirred at this temperature for 1 h. The reaction mixture was quenched with NH_4Claq (saturated solution) and the organic layer was separated, washed with brine, dried and concentrated in vacuo. The mixture was stirred at 0 °C for 5 minutes, then allowed to reach room temperature. Bis(1,1-dimethylethyl) dicarbonate was then added and the solution was left stirring overnight at room temperature under nitrogen. After quenching and work-up, the crude product purified by flash-chromatography NMR (^{1}H, $CDCl_3$): δ 11.95–12.02 (m, 1H), 4.07 (br s, 2H), 3.77–3.82 (m, 3H), 3.58 (t, 2H), 2.34–2.43 (m, 2H), 1.46–1.50 (m, 9H).

4.3.2.2. N-linked heterocycles. The example reported for derivatives **22**/**23** find general application for all the other N-linked heterocycles. Sodium hydride 60% dispersed in mineral oil was added at 0 °C to a solution of 3-(trifluoromethyl)-1*H*-pyrazole in dry DMF. The mixture was stirred at this temperature for 15 min, then for an additional 5 min at room temperature. A solution of derivative **45** in dry DMF (2 ml) was then added and the mixture was stirred at 50 °C for 3 h 30 min. After cooling to RT, a saturated NH_4Cl solution was added and the mixture was extracted with Et_2O (2×). The organic phase was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by Silica gel flash chromatography (gradient ethyl acetate in cyclohexane from 5 to 40%) to give the intermediate BOC protected compound **22** (colourless oil, R_f = 0.44, cyclohexane/ethyl acetate 8/2), MS (*m/z*): 434 $[MH-56]^+$; the intermediate BOC protected compound **23** (colorless oil, R_f = 0.33, cyclohexane/ethyl acetate 8/2); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 0.90 (d, 1H), 1.34 (s, 9H), 1.47–1.57 (m, 1H), 1.84–1.97 (m, 1H), 2.04–2.15 (m, 1H), 3.12–3.28 (m, 2H), 3.34–3.62 (m, 3H), 4.30 (d, 1H), 6.70 (d, 1H), 7.38 (dd, 1H), 7.60 (d, 1H), 7.67 (d, 1H), 7.87 (d, 1H); MS (*m/z*): 434 $[MH-56]^+$. Compounds were dissolved in CH_2Cl_2 and TFA was added. The reaction mixture was stirred at room temperature overnight. The volatiles were evaporated under reduced pressure and the residue purified by SCX, eluting first with MeOH and then with 2.0 N NH_3 in MeOH obtaining:

4.3.2.3. 6-(3,4-Dichlorophenyl)-1-{[5-(trifluoromethyl)-1Hpyrazol-1-yl]methyl}-3-azabicyclo[4.1.0]heptane (22). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (d, 1H), 1.48 (d, 1H), 1.89–2.09 (m, 2H), 2.70–2.86 (m, 2H), 2.95 (d, 1H), 3.18 (d, 1H), 3.59 (d, 1H), 3.98 (d, 1H), 6.58 (s, 1H), 7.17 (d, 1H), 7.35–7.43 (m, 2H), 7.56 (s, 1H). MS (*m/z*): 390 $[MH]^+$.

4.3.2.4. 6-(3,4-Dichlorophenyl)-1-{[3-(trifluoromethyl)-1Hpyrazol-1-yl]methyl}-3-azabicyclo[4.1.0]heptane (23). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.22 (d, 1H), 1.37 (d, 1H), 1.89–2.10 (m, 2H), 2.68–2.83 (m, 2H), 2.91 (d, 1H), 3.13 (d, 1H), 3.42 (d, 1H), 4.13 (d, 1H), 6.48 (s, 1H), 7.15 (d, 1H), 7.29–7.34 (m, 1H), 7.36–7.44 (m, 2H), NH not observed; MS (*m/z*): 390 $[MH]^+$.

4.3.3. C1-linked heterocycles

4.3.3.1. 1,1-Dimethylethyl -6-(3,4-dichlorophenyl)-1-formyl-3-azabicyclo[4.1.0]heptane-3-carboxylate (46). To a stirred solution of methyl derivative **44**[5,6] in dry CH_2Cl_2, at 0 °C and under a nitrogen atmosphere, bis(1,1-dimethylethyl) dicarbonate was added and the solution was left stirring overnight at room temperature under nitrogen. After quenching and work up, the compound was reacted with Dess–Martin Periodinane portion wise in CH_2Cl_2 at 0 °C. The reaction mixture was then warmed to rt, stirred for 3 h and was quenched with sat. $NaHCO_3$. Sodium thiosulphate (2 g in 5 ml of water) was added and the two phase system was stirred for 30 min. The organic layer was separated, dried and concentrated affording the title compound as yellow foam to give the title compound. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 8.50 (1H, s), 7.38–7.44 (2H, m), 7.12 (1H, dd), 4.31 (1H, br s), 3.34–3.96 (2H, m), 3.24 (1H, br s), 2.04–2.19 (2H, m), 1.99 (1H, d), 1.73 (1H, m), 1.46–1.55 (9H, m). MS (*m/z*): 370 $[MH]^+$ and 314 $[MH-56]^+$.

The aldehyde was reacted with Jones Reagent in acetone at 0 °C. After quenching and work up, to the acid intermediate carbonyl bis imidazole in ethyl acetate at rt. The mixture was stirred at rt for 1.5 h and then cooled to 0 °C in an ice-bath. Concentrated ammonium hydroxide was then added to give the intermediate amide **47**. This can be reacted according to literature procedures to prepare a wide range of heterocycles. To prepare sulphur containing heterocycles, the amide in THF at rt was added Lawesson's reagent. The mixture was heated to reflux temperature for 4 h and then slowly cooled to RT. After quenching and work up, the thioamide was reacted according to literature procedures.

4.3.4. C linked 'homologated' heterocycles

4.3.4.1. 1,1-Dimethylethyl -6-(3,4-dichlorophenyl)-1-ethenyl-3-azabicyclo[4.1.0]heptane-3-carboxylate (48). To a stirred suspension of methyltriphenylphosphonium bromide in THF at 0 °C, BuLi 1.6 M in hexane was added drop wise. The dark yellow reaction mixture was allowed to reach room temperature and stirred for 20 min. It was cooled to 0 °C and intermediate **46** in THF was added drop wise. The mixture was stirred at rt for 18 h. After quenching and work up, the residue was purified by chromatography on Silica gel the title compound as a colourless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.36–7.39 (1H, d), 7.31 (1H, d), 7.06 (1H, dd), 4.90–5.17 (3H, m), 3.89–4.20 (1H, m), 3.41–3.71 (2H, m), 3.27 (1H, ddd), 1.97–2.15 (2H, m), 1.51 (9H, s), 1.22–1.27 (2H, m); MS (*m/z*): 312 $[MH-56]^+$.

4.3.4.2. 6-(3,4-Dichlorophenyl)-3-{[(1,1-dimethylethyl)oxy]carbonyl}-3-azabicyclo[4.1.0]hept-1-yl)acetic acid (49). To a stirred solution **48** in THF at 0 °C, under nitrogen, borane tetrahydrofuran complex 1.0 M in THF was added drop wise. The ice bath was removed and the mixture was stirred at room temperature for 4 h. The mixture was cooled again to 0 °C and quenched with water, then NaOH 2.0 M and H_2O_2 (30% (w/w) were added. The resulting mixture was stirred for 0.5 h and then diluted with water and AcOEt. The organic layer was separated, dried and concentrated. As crude material, it was dissolved in acetone at 0 °C and Jones reagent (prepared dissolving chromium trioxide, 398 mg, in water, 0.7 ml, and sulphuric acid, 0.212 ml) was added drop wise; the mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched at 0 °C with 5 ml of isopropanol and stirred at this temperature for 10 min. The mixture was diluted with water and CH_2Cl_2; the organic layer dried and concentrated in vacuo to give **49** as a brown foam. HPLC: rt = 0.87 min; MS (*m/z*): 344 $[MH-56]^-$. This intermediate was dissolved in DMF under nitrogen at rt. DIPEA was added followed by TBTU. The mixture was stirred at room temperature for 30 min and then hesamethyl disilazane was added. The mixture was stirred at rt for 1.5 h. It was then quenched with water (10 ml) and diluted with diethyl ether (30 ml). After quenching and work up the intermediate 50 (as primary amide in this case) was obtained as brown foam. MS (*m/z*): 399 $[MH]^+$ and 343 $[MH-56]^+$. Appropriate heterocycles and sulphur containing heterocycles were prepared according to literature procedures as described above.

4.3.4.3. 6-(3,4-Dichlorophenyl)-1-(5-methyl-1,3-oxazol-2-yl)-3-azabicyclo[4.1.0]heptane (4). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.27 (1H, d), 7.20–7.24 (1H, d), 6.98 (1H, dd), 6.40 (1H, q), 3.95 (1H, d), 3.28 (1H, d), 2.76–2.93 (2H, m), 2.12 (1H, d), 2.05–

2.07 (4H, m), 1.89–1.98 (1H, m), 1.42–1.45 (1H, d); MS (*m/z*): 323 $[MH]^+$.

4.3.4.4. 6-(3,4-Dichlorophenyl)-1-(4-methyl-1,3-thiazol-2-yl)-3-azabicyclo[4.1.0]heptane (5). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.26–7.31 (1H, m), 7.18 (1H, d), 6.99 (1H, dd), 6.51 (1H, q), 3.89 (1H, d), 3.39 (1H, d), 2.82–2.99 (2H, m), 2.28 (3H, d), 1.91–2.13 (3H, m), 1.56 (1H, d); MS (*m/z*): 339 $[MH]^+$.

4.3.4.5. 6-(3,4-Dichlorophenyl)-1-(5-methyl-3-isoxazolyl)-3-azabicyclo[4.1.0]heptane (8). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.46 (d, 1H), 1.78 (d, 1H), 1.89–2.12 (m, 2H), 2.22 (s, 3H), 2.76–2.94 (m, 2H), 3.30 (d, 1H), 3.68 (d, 1H), 5.34 (s, 1H), 6.98 (dd, 1H), 7.20–7.26 (m, 2H); MS (*m/z*): 323 $[MH]^+$.

4.3.4.6. 6-(3,4-Dichlorophenyl)-1-(3-methyl-1,2,4-oxadiazol-5-yl)-3-azabicyclo[4.1.0]heptane (9). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.64 (d, 1H), 1.90–2.03 (m, 1H), 2.02–2.18 (m, 1H), 2.24 (d, 1H), 2.25 (s, 3H), 2.76–2.99 (m, 2H), 3.34 (d, 1H), 4.01 (d, 1H), 6.98 (dd, 1H), 7.24–7.30 (m, 2H); MS (*m/z*): 324 $[MH]^+$.

4.3.4.7. 6-(3,4-Dichlorophenyl)-1-[(4-methyl-1,3-thiazol-2-yl)methyl]-3-azabicyclo[4.1.0]heptane (10). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.38–7.41 (2H, m), 7.15 (1H, dd), 6.71–6.75 (1H, m), 3.07–3.16 (2H, m), 3.02 (1H, d), 2.71–2.83 (2H, m), 2.41 (3H, s), 2.22 (1H, d), 1.99–2.08 (1H, m), 1.90–1.98 (1H, m), 1.29 (1H, d), 1.15–1.19 (1H, m); MS (*m/z*): 353 $[MH]^+$.

4.3.4.8. 6-(3,4-Dichlorophenyl)-1-[(4-methyl-1,3-oxazol-2-yl)methyl]-3-azabicyclo[4.1.0]heptane (11). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.34–7.38 (2H, m), 7.24 (1H, d), 7.12 (1H, dd), 3.07–3.19 (2H, m), 2.71–2.82 (2H, m), 2.58 (1H, d), 2.10–2.17 (4H, m), 1.88–2.05 (2H, m), 1.24 (1H, d), 1.10 (1H, d); MS (*m/z*): 337 $[MH]^+$.

4.3.4.9. 6-(3,4-Dichlorophenyl)-1-[(4-methyl-1,3-oxazol-5-yl)methyl]-3-azabicyclo[4.1.0]heptane (12). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.69 (1H, s), 7.38–7.43 (2H, m), 7.14 (1H, dd), 2.99 (2H, s), 2.76–2.86 (1H, m), 2.65–2.74 (1H, m), 2.55 (1H, d), 1.84–2.07 (6H, m), 1.19 (1H, d), 1.05 (1H, d); MS (*m/z*): 337 $[MH]^+$.

4.3.4.10. 6-(3,4-Dichlorophenyl)-1-[(5-methyl-1Hpyrazol-1-yl)methyl]-3-azabicyclo[4.1.0]heptane (14, 15). Racemate **13** was submitted to chiral HPLC (Preparative conditions: chiral column Chiralpak AD-H, eluent A: *n*-hexane; B: 2-propanol 90/10 v/v, flow rate 14 ml/min, detection UV 230 nm. Analytical conditions: chiral column Chiralpak AD-H, eluent A: *n*-hexane; B: 2-propanol 90/10 v/v, flow rate 1 ml/min, DAD 210–340 nm) obtaining: **14** rt 6.18 min and **15 rt** 7.31 min.

^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.33–7.43 (2H, m), 7.10–7.21 (2H, m), 5.97 (1H, s), 4.01 (1H, d), 3.25 (1H, d), 3.12 (1H, d), 2.91 (1H, d), 2.65–2.82 (2H, m), 2.25 (3H, s), 1.82–2.07 (2H, m), 1.36 (1H, d), 1.16 (1H, d); MS (*m/z*): 336 $[MH]^+$.

4.3.4.11. 6-(3,4-Dichlorophenyl)-1-[(5-methyl-1Hpyrazol-1-yl)methyl]-3-azabicyclo[4.1.0]heptane (16). ^{1}H NMR (DMSO, 400 MHz) δ ppm 8.78 (2H, br s), 7.76 (1H, d), 7.57–7.64 (1H, m), 7.47 (1H, dd), 7.35 (1H, d), 6.00 (1H, s), 4.00 (1H, d), 3.63 (1H, d), 3.10–3.23 (2H, m), 2.93–3.04 (1H, m), 2.77–2.89 (1H, m), 2.05–2.29 (2H, m), 2.00 (3H, s), 1.55 (1H, d), 1.35 (1H, d); MS (*m/z*): 336 $[MH]^+$.

4.3.4.12. 6-(3,4-Dichlorophenyl)-1-[(4-methyl-1Hpyrazol-1-yl)methyl]-3-azabicyclo[4.1.0]heptane (18). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 7.36–7.42 (2H, m), 7.27 (1H, s), 7.16 (1H, dd), 7.00 (1H, s), 4.02 (1H, dd), 3.26 (1H, d), 3.13 (1H, d), 2.90 (1H, d), 2.67–2.82 (2H, m), 1.96–2.08 (4H, m), 1.86–1.97 (1H, m), 1.37 (1H, d), 1.17 (1H, dd); MS (*m/z*): 336 $[MH]^+$.

4.3.4.13. 6-(3,4-Dichlorophenyl)-1-[(3,5-dimethyl-1Hpyrazol-1-yl)methyl]-3-azabicyclo[4.1.0]heptane (19). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 7.35–7.43 (2H, m), 7.16 (1H, dd), 5.71–5.78 (1H, m), 3.87 (1H, d), 3.16–3.23 (2H, m), 2.89 (1H, d), 2.69–2.85 (2H, m), 2.22 (3H, s), 2.07 (3H, s), 1.97–2.07 (1H, m), 1.87–1.95 (1H, m), 1.38–1.42 (1H, m), 1.16–1.19 (1H, m); MS (*m/z*): 350 $[MH]^+$. Retention times for **20** and for **21**: rt = 5.68 min and rt = 6.90 min, respectively.

4.3.4.14. 6-(3,4-Dichlorophenyl)-1-{[5-(trifluoromethyl)-1Hpyrazol-1-yl]methyl}-3-azabicyclo[4.1.0]heptane (22). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (d, 1H), 1.48 (d, 1H), 1.89–2.09 (m, 2H), 2.70–2.86 (m, 2H), 2.95 (d, 1H), 3.18 (d, 1H), 3.59 (d, 1H), 3.98 (d, 1H), 6.58 (s, 1H), 7.17 (d, 1H), 7.35–7.43 (m, 2H), 7.56 (s, 1H); MS (*m/z*): 390 $[MH]^+$.

4.3.4.15. 6-(3,4-Dichlorophenyl)-1-{[3-(trifluoromethyl)-1Hpyrazol-1-yl]methyl}-3-azabicyclo[4.1.0]heptane (23). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.22 (d, 1H), 1.37 (d, 1H), 1.89–2.10 (m, 2H), 2.68–2.83 (m, 2H), 2.91 (d, 1H), 3.13 (d, 1H), 3.42 (d, 1H), 4.13 (d, 1H), 6.48 (s, 1H), 7.15 (d, 1H), 7.29–7.34 (m, 1H), 7.36–7.44 (m, 2H); MS (*m/z*): 390 $[MH]^+$.

4.3.4.16. 6-(3,4-Dichlorophenyl)-3-azabicyclo[4.1.0]hept-1-yl]methyl}-1Hpyrazole-5-carbonitrile (24). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20 (d, 1H), 1.60 (d, 1H), 1.90–2.11 (m, 2H), 2.68–2.92 (m, 3H), 3.22 (d, 1H), 3.53 (d, 1H), 4.27 (d, 1H), 6.77 (s, 1H), 7.20 (d, 1H), 7.43 (m, 2H), 7.58 (s, 1H); MS (*m/z*): 347 $[MH]^+$.

4.3.4.17 6-(3,4-Dichlorophenyl)-3-azabicyclo[4.1.0]hept-1-yl]methyl}-1Hpyrazole-3-carbonitrile (**25**).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.23 (d, 1H), 1.38 (d, 1H), 1.90–2.10 (m, 2H), 2.68–2.85 (m, 2H), 2.91 (d, 1H), 3.13 (d, 1H), 3.43 (d, 1H), 4.13 (d, 1H), 6.65 (d, 1H), 7.15 (d, 1H), 7.32 (d, 1H), 7.38–744 (m, 2H); MS (*m/z*): 347 $[MH]^+$.

4.3.4.18. 6-(3,4-Dichlorophenyl)-1-{[5-methyl-3-(trifluoromethyl)-1Hpyrazol-1-yl]methyl}-3-azabicyclo[4.1.0]heptane (26). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.33–7.38 (m, 2H) 7.13 (dd, 1H) 6.22 (s, 1H) 3.81 (d, 1H) 3.44 (d, 1H) 3.22 (d, 1H) 2.89 (d, 1H) 2.70–2.82 (m, 2H) 2.09 (s, 3H) 1.87–2.06 (m, 2H) 1.37 (d, 1H) 1.19 (d, 1H); MS (*m/z*): 404 $[MH]^+$.

4.3.4.19. 6-(3,4-Dichlorophenyl)-1-{[3-methyl-5-(trifluoromethyl)-1Hpyrazol-1-yl]methyl}-3-azabicyclo[4.1.0]heptane (27). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.43 (d, 1H) 7.38 (d, 1H) 7.18 (dd, 1H) 6.35 (br s, 1H) 3.90 (d,1H) 3.50–3.53 (m, 2H) 3.14 (d, 1H) 2.95 (d, 1H) 2.74–2.81 (m, 2H) 2.30 (s, 3H) 1.88–2.08 (m, 2H) 1.45 (d, 1H) 1.18 (d, 1H); MS (***m/z***): 404 $[MH]^+$.

4.3.4.20. 6-(3,4-Dichlorophenyl)-1-{[3-(1,1-dimethylethyl)-1Hpyrazol-1-yl]methyl}-3-azabicyclo[4.1.0]heptane (28). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.46 (d, 1H) 7.37–7.42 (m, 1H) 7.31–7.36 (m, 1H) 7.04 (d, 1H) 6.05 (d, 1H) 3.98 (d, 1H) 3.75 (d, 1H) 3.55 (d, 1H) 3.24–3.35 (m, 1H) 3.11 (d, 1H) 2.89–3.01 (m, 1H) 2.37–2.49 (m, 1H) 2.20–2.29 (m, 1H) 1.53 (d, 1H) 1.34 (d, 1H) 1.31 (s, 9H); MS (*m/z*): 378 $[MH]^+$.

4.3.4.21. 6-(3,4-Dichlorophenyl)-1-(1Hpyrrol-1-ylmethyl)-3-azabicyclo[4.1.0]heptane (29). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.43 (2H, dd), 7.19 (1H, dd), 6.58 (2H, t), 6.10–6.15 (2H, m), 3.88 (1H, dd), 3.01–3.09 (2H, m), 2.90 (1H, d), 2.69–2.83 (2H, m),

1.92–2.12 (2H, m), 1.36 (1H, d), 1.19–1.25 (1H, m); MS (*m/z*): 321[MH]$^+$.

4.3.4.22. 6-(3,4-Dichlorophenyl)-3-azabicyclo[4.1.0]hept-1-yl]methyl}-1Hpyrrole-2-carbonitrile (30). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.43 (1H, d), 7.41 (1H, d), 7.17 (1H, dd), 6.78–6.80 (1H, m), 6.76 (1H, dd), 6.16 (1H, dd), 4.08 (1H, dd), 3.18 (1H, d), 3.04 (1H, d), 2.81–2.89 (2H, m), 2.64–2.74 (1H, m), 2.00–2.10 (1H, m), 1.89–1.97 (1H, m), 1.48 (1H, d), 1.25 (1H, dd); MS (*m/z*): 346 [MH]$^+$.

4.3.4.23. 6-(3,4-Dichlorophenyl)-3-azabicyclo[4.1.0]hept-1-yl]methyl}-1Hpyrrole-3-carbonitrile (31). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 7.45–7.39 (m, 2H) 7.16 (d, 1H) 7.08 (s, 1H) 6.56 (s, 1H) 6.40 (s, 1H) 3.87 (d, 1H) 3.07 (d, 1H) 2.97 (d, 1H) 2.88–2.68 (m, 3H) 2.09–1.92 (m, 2H) 1.29 (dd, 2H); MS (*m/z*): 346 [MH]$^+$.

4.3.4.24. 6-(3,4-Dichlorophenyl)-1-[(3-methyl-1Hpyrrol-1-yl)methyl]-3-azabicyclo[4.1.0]heptane (32). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 7.43–7.40 (m, 2H) 7.15 (d, 1H) 6.45 (s, 1H) 6.33 (s, 1H) 5.93 (s, 1H) 3.77 (d, 1H) 3.02–2.91 (m, 3H) 2.80–2.70 (m, 2H) 2.08 (s, 3H) 2.03–1.92 (m, 2H) 1.31 (d, 1H) 1.18 (d, 1H); MS (*m/z*): 335 [MH]$^+$.

4.3.4.25. 6-(3,4-Dichlorophenyl)-1-[(2,5-dimethyl-1Hpyrrol-1-yl)methyl]-3-azabicyclo[4.1.0]heptane (33). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.41–7.50 (2H, m), 7.22 (1H, dd), 5.75 (2H, s), 3.87 (1H, d), 2.61–2.90 (5H, m), 2.16–2.24 (6H, m), 2.02–2.12 (1H, m), 1.92–2.01 (1H, m), 1.25–1.39 (2H, m); MS (*m/z*): 349 [MH]$^+$.

4.3.4.26. 6-(3,4-Dichlorophenyl)-1-[(2,4-dimethyl-1Hpyrrol-1-yl)methyl]-3-azabicyclo[4.1.0]heptane (34). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.21 (dd, 1H), 1.32 (d, 1H), 1.89–1.97 (m, 1H), 1.98–2.06 (m, 4H), 2.06–2.10 (m, 3H), 2.66–2.76 (m, 1H), 2.78–2.86 (m, 2H), 2.91–3.00 (m, 2H), 3.74 (dd, 1H), 5.63–5.68 (m, 1H), 6.26–6.30 (m, 1H), 7.16 (dd, 1H), 7.37–7.43 (m, 2H); MS (*m/z*): 349 [MH]$^+$.

4.3.4.27. 6-(3,4-Dichlorophenyl)-1-(1H1,2,4-triazol-1-ylmethyl)-3-azabicyclo[4.1.0]heptane (35). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 7.92 (1H, s), 7.92 (1H, s), 7.42 (1H, d), 7.41 (1H, d), 7.16 (1H, dd), 4.12 (1H, d), 3.41 (1H, d), 3.14 (1H, d), 2.95 (1H, d), 2.68–2.83 (2H, m), 1.99–2.09 (1H, m), 1.90–1.99 (1H, m), 1.37 (1H, d), 1.22 (1H, d); MS (*m/z*): 323 [MH]$^+$.

4.3.4.28. 6-(3,4-Dichlorophenyl)-1-[(3,5-dimethyl-1H1,2,4-triazol-1-yl)methyl]-3-azabicyclo[4.1.0]heptane (36). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.38–7.44 (m, 2H) 7.15 (dd, 1H) 3.86 (d, 1H) 3.23 (d, 1H) 3.15 (d, 1H) 2.98 (d, 1H) 2.69–2.86 (m, 2H) 2.34 (s, 3H) 2.24 (s, 3H) 1.99–2.09 (m, 1H) 1.89–1.97 (m, 1H) 1.40 (d, 1H) 1.20 (s, 1H); MS (*m/z*): 351 [MH]$^+$ rt = 17.8 min and rt = 21.6 for derivatives **37** and **38** respectively.

4.3.4.29. 6-(3,4-Dichlorophenyl)-1-{[5-(1-methylethyl)-1Htetrazol-1-yl]methyl}-3-azabicyclo[4.1.0]heptane (39). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.38 (d, 1H) 7.36 (d, 1H) 7.11 (dd, 1H) 3.96 (d, 1H) 3.63 (d, 1H) 3.25 (d, 1H) 2.98 (d, 1H) 2.78–2.87 (m, 2H) 2.71–2.79 (m, 2H) 1.93–2.09 (m, 2H) 1.42 (d, 1H) 1.26–1.32 (m, 7H); MS (*m/z*): 366 [MH]$^+$.

4.3.4.30. 6-(3,4-Dichlorophenyl)-1-{[5-(1-methylethyl)-2H-tetrazol-2-yl]methyl}-3-azabicyclo[4.1.0]heptane (40). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.45 (br s, 1H) 7.40 (d, 1H) 7.15 (dd, 1H) 4.40 (d, 1H) 3.88 (d, 1H) 3.2 (m, 2H) 2.99 (d, 1H) 2.76 (m, 2H) 2.0 (m, 2H) 1.46 (d, 1H) 1.39 (dd, 1.66 Hz, 6H) 1.18 (d, 1H), MS (*m/z*): 366 [MH]$^+$.

4.3.4.31. 6-(3,4-Dichlorophenyl)-3-azabicyclo[4.1.0]hept-1-yl]methyl}-1Hindole (41). 1H NMR (400 MHz, $CDCl_3$) δ ppm 1.22 (d, 1H), 1.45 (d, 1H), 1.95–2.12 (m, 2H), 2.69–2.90 (m, 3H), 3.15 (d, 1H), 3.25 (d, 1H), 4.21 (d, 1H), 6.49 (d, 1H), 7.02–7.30 (m, 5H), 7.43–7.50 (m, 2H), 7.60 (d, 1H); MS (*m/z*): 371 [MH]$^+$.

4.3.4.32. 6-(3,4-Dichlorophenyl)-3-azabicyclo[4.1.0]hept-1-yl]methyl}-1Hindazole (42). 1H NMR (400 MHz, $CDCl_3$) δ ppm 1.17 (d, 1H), 1.51 (d, 1H), 1.92–2.09 (m, 2H), 2.71–2.84 (m, 2H), 2.88 (d, 1H), 3.25 (d, 1H), 3.62 (d, 1H), 4.30 (d, 1H), 7.13 (t, 1H), 7.17 (d, 1H), 7.22 (dd, 1H), 7.31 (t, 1H), 7.39 (d, 1H), 7.46 (d, 1H), 7.71 (d, 1H), 7.99 (s, 1H); MS (*m/z*): 372 [MH]$^+$.

4.3.4.33. 6-(3,4-Dichlorophenyl)-3-azabicyclo[4.1.0]hept-1-yl]methyl}-2H-indazole (43). 1H NMR (400 MHz, $CDCl_3$) δ ppm 1.25 (d, 1H), 1.51 (d, 1H), 1.91–2.12 (m, 2H), 2.69–2.85 (m, 2H), 2.93 (d, 1H), 3.20 (d, 1H), 3.65 (d, 1H), 4.40 (d, 1H), 7.07 (t, 1H), 7.19 (dd, 1H), 7.24–7.31 (m, 1H), 7.39–7.47 (m, 2H), 7.62 (d, 1H), 7.69 (d, 1H), 7.79 (s, 1H); MS (*m/z*): 372 [MH]$^+$.

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