Research paper

New β-arylchalcogeno amines with procognitive properties targeting Carbonic Anhydrases and Monoamine Oxidases

Gustavo Provensi [a,**], Alessia Costa [b], Barbara Rani [b], Maria Vittoria Becagli [b], Fabio Vaiano [b], Maria Beatrice Passani [b], Damiano Tanini [c], Antonella Capperucci [c], Simone Carradori [d], Jacobus P. Petzer [e], Anél Petzer [e], Daniela Vullo [f], Gabriele Costantino [g], Patrizio Blandina [a], Andrea Angeli [f,g,*], Claudiu T. Supuran [f]

[a] Department of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Viale G. Pieraccini 6, 50139, Florence, Italy
[b] Department of Health Sciences, University of Florence, Viale Pieraccini 6 Florence, Florence, Italy
[c] Department of Chemistry "Ugo Schiff", University of Florence, Via Della Lastruccia 3-13, I-50019, Sesto Fiorentino, Italy
[d] Department of Pharmacy, "G. D'Annunzio" University of Chieti-Pescara, Via Dei Vestini 31, 66100, Chieti, Italy
[e] Pharmaceutical Chemistry and Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa
[f] NEUROFARBA Department, Sezione di Scienze Farmaceutiche, University of Florence, Via Ugo Schiff 6, 50019, Sesto Fiorentino, Florence, Italy
[g] Department of Food and Drug, University of Parma, Parco Area Delle Scienze, 27/A, 43124, Parma, Italy

ARTICLE INFO

ABSTRACT

Cognitive deficits are enduring and disabling symptoms for many patients with severe mental illness, and these impairments are inadequately addressed by current medications. In this study, we reported the synthesis of β-arylchalcogeno amines bearing sulfurated, selenated, and tellurated moieties (2–4) which are structurally related to amphetamine with good activation properties for Carbonic Anhydrases (CAs) isoforms present in the cortical and hippocampal brain structures (hCA IV and hCA XIV). In addition, these compounds showed selective inhibition against the Monoamine oxidase (MAO) A isoform. In vivo evaluation of two derivatives (2a and 3a) revealed procognitive effects in the object recognition and social discrimination tests. Interestingly, these compounds, despite having a similar structure to amphetamine, did not caused hypophagia or hyperlocomotion, two effects often observed following the administration of amphetamine-like drugs. In this context, β-arylchalcogeno amines may have utility for improving the symptoms of cognitive decline associated with neurodegenerative and psychiatric diseases such as attention deficit disorder, Parkinson's disease-related cognitive dysfunction and cognitive disorders associated with depression.

1. Introduction

Cognition comprises of multiple high-level mental abilities such as perception, attention, reasoning, planning, problem solving, decision-making, language, memory which are essential for the proper functioning of an individual [1]. "Cognitive impairments" is an umbrella term used to define dysfunctions in one or more of these processes leading to problems in general adjustment, emotional and social functioning, and well-being [2]. Deficits in brain networks that support cognitive abilities are among the most prevalent complications in many neurodegenerative and psychiatric disorders. Indeed, most patients with different conditions such as Alzheimer disease, Parkinson disease, Lewy body dementia, Huntington disease, HIV dementia depression, schizophrenia, chronic alcohol or substance abuse, brain tumors, epilepsy, multiple sclerosis, manifest cognitive deficits, often at the beginning of the disease course [3]. These cognitive disfunctions are largely separate from the classical symptoms of the disorders. Therefore, they often persist even in the face of symptomatically effective treatment [4].

There is a growing body of evidence demonstrating the complex and multifactorial nature of cognitive impairments [5,6]. In this context, we

---

* Corresponding author. NEUROFARBA Department, Sezione di Scienze Farmaceutiche, University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy.
** Corresponding author. Department of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Viale G. Pieraccini 6, 50139, Florence, Italy.
E-mail addresses: gustavo.provensi@unifi.it (G. Provensi), andrea.angeli@unifi.it (A. Angeli).

hypothesized that compounds acting synergistically on multiple targets could potentially translate into a novel polypharmacological tools to treat cognitive impairments. Building on this concept, our efforts focused on the design and synthesis of new compounds with a multi-target profile: Carbonic Anhydrase (CA, EC 4.2.1.1) activators and Monoamine Oxidase (MAO, EC 1.4.3.4) inhibitors.

CAs are ubiquitously expressed enzymes involved in several physiological processes, including cognition [7,8]. Modulation of the activities of CAs activity was shown to impact animal's learning in different models evaluating spatial [9,10], aversive [11] and recognition [12] memories. Recently, we demonstrated that infusion of activators/inhibitors of the CAs in specific brain areas such as the hippocampus and the frontal cortex impacts on fear memory extinction [13] and social discrimination [14]. Moreover, a key role for brain associated isoform hCA VII in protecting cells against oxidative stress was reported making it an excellent target [15]. A growing body of evidence indicates that disturbances of antioxidant defense systems and the presence of oxidative stress may play a role in the biochemical mechanisms underlying cognitive disorders [16]. Oxidative stress in the brain is also decreased by inhibition of mitochondrial enzymes such as Monoamine Oxidase (MAO, EC 1.4.3.4). MAOs catalyze the oxidative deamination of biogenic and xenobiotic amines and have an important role in the metabolism of neuroactive and vasoactive amines in the central nervous system (CNS) and peripheral tissues [17]. It has been observed in several studies that MAOs, in particular the MAO-A isoform, are involved in neurological and psychiatric disorders [18]. Recent data obtained in animals and in humans suggest that both MAO-A and MAO-B inhibitors present cognitive enhancing properties of possible interest in the treatment of cognitive disorders [17]. Based on our recent report showing that amphetamine as well as other structurally-related amines such as methamphetamine, phentermine, mephentermine, and chlorphenteramine, potently activate human CA isoforms [19], here we developed a series of β-arylchalcogeno amines 2, 3, and 4, bearing sulphurated, selenated, and tellurated moieties showing antioxidant propriety useful to solve oxidative stress problems involved in neuropsychiatric disorders. These compounds showed potent CA activating, in particular hCA IV, VII and XIV isoforms, selective MAO-A inhibiting property. Two derivatives (**2a** and **3a**) facilitated the consolidation of recognition memory *in vivo*. Interestingly, these compounds, despite having a similar structure to amphetamine, did not caused hypophagia or hyperlocomotion, which is a positive feature for the potential pharmacotherapeutic profile of these compounds.

2. Results and discussion

2.1. Design and synthesis

At the initial stage of the project, we chose the well-studied amphetamine (AMP, Fig. 1) scaffold due to its multiple psychotropic effects and having recently been discovered as an activator of several CA

isoforms [19]. Moreover, a remarkable feature of the AMP skeleton is that it is possible to produce drastic changes in its pharmacodynamics with minimal structural variations. This leads to compounds with useful activities that differentially interact with different target proteins such as two MAO isoforms [20]. Indeed, to obtain selective inhibitors of MAO-A, as its abnormal activity is associated with several neuropsychiatric disorders [21], the presence of a methyl group on the α-carbon atom is essential [20]. Finally, we evaluated the possibility of lengthening the side-chain with a chalcogenide atom such as sulphur, selenium and tellurium in order to evaluate the antioxidant proprieties and fight the ROS species associated to neuropsychiatric disorders (Fig. 1).

β-Arylchalcogeno amines 2, 3, and 4 were prepared through the nucleophilic ring opening reaction of the *N*–H inactivated 2-methylaziridine **1** with suitable sulphur-, selenium-, and tellurium-centered nucleophiles. Amino-substituted sulphides **2a,b**, selenides **3a,b**, and the telluride **4a** were efficiently synthesised from the corresponding silyl chalcogenides and the aziridine **1** by a fluoride-induced silicon-mediated procedure developed by some of us (Scheme 1) [22]. On the other hand, differently substituted thiolates and selenolates – *in situ* generated upon deprotonation of the corresponding thiols and selenols with KOH – were employed for the synthesis of sulfides **2c,d** and selenides **3c-g** (Scheme 2) [23].

Similarly, β-aryltelluro-amines **4b-d** were obtained through the reaction of aryltellurolates with the aziridine **1**. Tellurolates were readily generated upon reduction of the corresponding ditellurides with NaBH₄ in ethanol (Scheme 3) [23]. Notably, all these ring opening reactions occurred with high regioselectivity, leading to the formation of the sole product arising from the nucleophilic attack onto the less hindered carbon of the three-membered heterocycle.
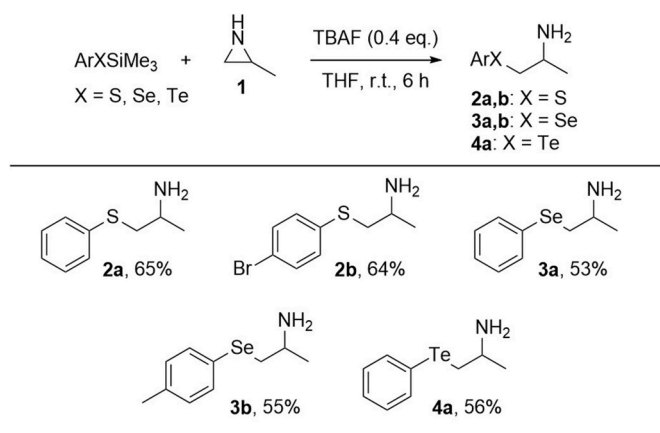
Scheme 1. Synthesis of β-arylchalcogeno amines *via* ring opening reaction of aziridines with silyl chalcogenides.
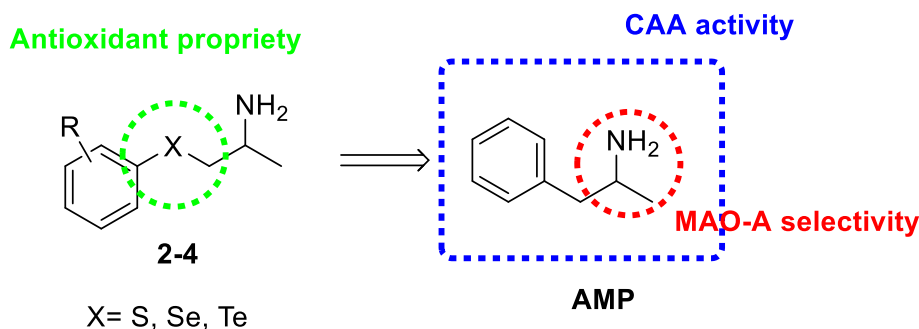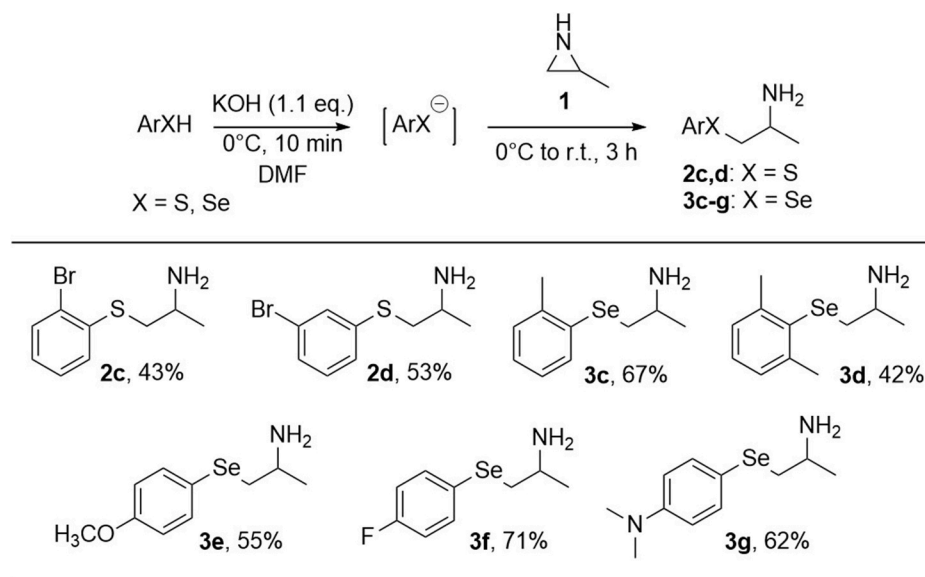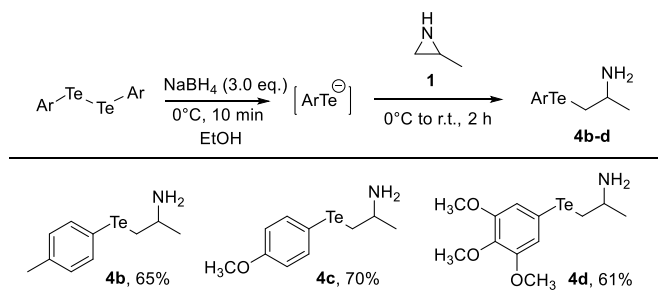

Fig. 1. General structure of novel hybrid CAAs.

Scheme 2. Synthesis of β-arylthio- and β-arylseleno-amines *via* ring opening reaction of aziridines with thiolates and selenolates.

Scheme 3. Synthesis of β-aryltelluro-amines *via* ring opening reaction of aziridines with tellurolates.

3. Carbonic anhydrase activation

All compounds (**2–4**) are tested *in vitro* for their activity against all catalytically active human CA isoforms I-XIV by means of the stopped-flow carbon dioxide hydration assay [24]. Their activities are compared to D-phenylalanine (D-Phe) and AMP as outlined in Table 1.

The following structure–activity relationship (SAR) can be observed regarding the activation data of Table 1:

i) The widely expressed isoform hCA II was not activated by these amines except for compound **3g** which showed an activation constant of 45.1 μM. On the other hand, the second most abundant isoform hCA I was moderately activated by several compounds studied here. It is interesting to note the difference in potency among the analogue compounds **3e** and **4c** where the simple replacement of one atom of selenium with tellurium drastically increased the potency of activation (K_A 21.4 μM and 4.6 μM, respectively). Keeping in mind the activity of AMP, that shows no activity against this isoform, the addition of one chalcogen atom in the AMP structure led to the activation of hCA I and the replacement of one atom of sulphur for compound **2a** with one of selenium (**3a**) or tellurium (**4a**) did not change significantly the activation constants (for **2a** K_A 11.8 to 9.9 μM for **3a** and **4a**) proving to be essential for the activity against hCA I.

ii) Other cytosolic isoforms such as hCA III, hCA VII and hCA XIII showed different activation profiles with these compounds. In

Table 1

Activation of human CA isoforms I-XIV with compounds 2–4, D-Phe and AMP by a stopped flow CO$_2$ hydrase assay [24].

K_A (μM)[a]

Cmp	hCA I	hCA II	hCA III	hCA IV	hCA VA	hCA VB	hCA VI	hCA VII	hCA IX	hCA XII	hCA XIII	hCA XIV
2a	11.8	>100	26.8	7.1	12.4	18.7	70.9	23.4	74.8	43.3	82.4	9.8
2b	7.7	>100	5.4	7.4	12.4	22.7	61.6	14.8	63.3	72.9	54.4	3.8
2c	9.5	>100	70.8	2.4	13.3	12.8	55.9	23.2	56.6	46.7	90.9	3.6
2d	13.5	>100	91.7	2.2	12.9	11.5	30.9	11.4	77.9	87.9	49.3	2.9
3a	9.9	>100	14.3	8.6	14.7	26.3	57.2	20.6	49.6	59.5	58.6	9.9
3b	5.2	>100	20.3	2.2	9.1	15.7	30.5	10.4	82.5	89.7	68.6.	8.3
3c	12.1	>100	54.1	2.7	20.0	23.7	71.6	14.2	83.3	51.8	49.3	4.7
3d	6.0	>100	90.8	9.5	20.9	22.7	31.4	21.4	81.2	78.2	83.3	1.1
3e	21.4	>100	66.8	8.7	20.3	25.8	46.4	23.3	73.2	68.8	60.4	8.7
3f	19.6	>100	13.1	6.1	13.1	20.2	48.5	13.4	22.8	90.6	46.9	2.6
3g	22.1	45.1	72.6	9.4	14.0	21.8	76.5	12.1	70.1	58.5	44.1	6.7
4a	9.9	>100	59.7	5.9	14.2	21.7	73.1	41.2	22.2	52.5	49.7	3.2
4b	8.2	>100	73.5	5.3	4.0	15.7	23.6	21.9	74.4	80.4	82.7	2.4
4c	4.6	>100	77.3	8.2	3.3	22.5	37.8	44.9	47.9	52.8	38.2	9.5
4d	9.5	>100	60.6	4.4	9.4	29.5	69.2	14.3	85.2	32.7	58.3	1.2
AMP	>150	>150	87.7	0.094	0.81	2.56	>150	0.91	>150	0.64	24.1	9.15
D-Phe	86	0.035	15.4	49.3	4.63	0.072	16	9.74	9.3	0.37	0.051	7.21

[a] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5–10% of the reported values).

particular, hCA III was observed to be moderately activated with constant values spanning between 5.4 μM and 91.7 μM. All the chalcogen analogues of AMP (2a, 3a and 4a) were more active (26.8, 14.3, 59.7 and 87.7 μM, respectively). In addition, when sulphur (2a) is replaced by selenium (3a), the activity increased nearly 2-fold; instead, when replaced by tellurium (4a), the activity decreased 4-times. Regarding the substitutions for compounds 2a-d, the best activity is observed for *para* substitution as for 2b (K_A 5.4 μM). On the other hand, substitution in *ortho* (2c) and *meta* (2d) positions proved to be deleterious for the activity reaching the high micromolar range values (K_A 70.8 and 91.7 μM). Regarding hCA VII, all compounds showing less activity than AMP and the replacement of chalcogen atom in its analogues (2a, 3a and 4a) did not show significant differences in terms of potency with K_A ranging from 20.6 to 41.2 μM. Regarding hCA XIII, the different ring substitutions resulted in no significant differences in power modulation, only the replacement of chalcogen atom for the analogue of AMP (2a, 3a and 4a) we observed a slight increase in the potency of activation.

iii) The mitochondrial isoforms hCA VA and hCA VB were moderately activated with K_As ranging from 3.3 μM to 29.5 μM. Regarding hCA VA, the best activators were compounds with tellurium atoms 4b-4d with K_As of 4.0, 3.3 and 9.4 μM, respectively. It is interesting to note that these compounds (4b-4d) showed a selectivity ratio of over 3-fold for hCA VA with respect to hCA VB and compound 4c was nearly 7-times more selective. On the other hand, the constant values against hCA VB were in the same range for all compounds studied here, irrespective of the substituents or chalcogen atom. These compounds remained, in any case, less effective on mitochondrial hCAs compared to the psychotropic amine AMP.

iv) Similar to hCA XII and XIII, the secreted isoform hCA VI, was weakly activated by compounds 2–4 with activation values in the high micromolar range (K_As 23.6–76.5 μM). However, all compounds had better activation constants compared to the reference compound AMP ($K_A > 150$ μM), suggesting that the addition of an atom in the scaffold enhanced the activity.

v) The membrane bound isoforms hCA IV and hCA XIV were the most activated isoforms by compounds 2–4 with a K_A in the low micromolar range. For hCA XIV the best activation values were observed the best activation values with a K_A of 1.1 μM for derivative 3d and 1.2 μM for 4d where, in both cases, bulky substituents proved to be essential to improve the potency. When comparing sulphur to tellurium substitution for the AMP analogues (2a, 3a and 4a), an approximately 3-fold increase in power for derivative 4a compared to compounds 2a and 3a (3.2 μM for 4a than 9.8/9.9 μM 2a/3a). A comparable activation profile can be observed for hCA IV, although less significantly. Interestingly, among these two isoforms, the addition of a chalcogen atom led to significant changes in CA activation compared to AMP. In fact, for hCA IV there was a drastic power decrease in activation compared to AMP; on the contrary, for hCA XIV we observed an increase in potency with respect to AMP. Regarding the membrane isoform, hCA XII, the addition of a chalcogen atom decreased the activation potency nearly 10-fold compared to AMP. No significant variations in potency were observed among the different chalcogen atoms and substituents. On the other hand, the addition of one atom to the AMP scaffold increased the activity against hCA IX compared to AMP and, in addition, substitution of sulphur (2a) for tellurium (4a) we observed an increase of over three times in activity.

3.1. MAO inhibition

The β-arylchalcogeno amine derivatives (2–4) were investigated as

potential inhibitors of recombinant human MAO-A and MAO-B [25]. Kynuramine was used as enzyme substrate for both MAO isoforms and is oxidized by the MAOs to yield 4-hydroxyquinoline, which was measured and quantitated by fluorescence spectrophotometry [26]. The IC_{50} values for the inhibition of the MAOs are presented in Table 2.

From the inhibition data it is evident that most compounds are selective inhibitors of MAO-A compared to MAO-B, which may be due to the methyl at the α-carbon position as mentioned above. Regarding the MAO-A inhibition, the sulphur derivative 2c, with bromine substituted on the *ortho* position, showed the best inhibition activity with an IC_{50} of 0.724 μM. On the other hand, placing the bromine on the *meta* position, led to the weakest MAO-A inhibition among the sulphur derivatives 2a-d. When considering the organoselenium derivatives 3a-g, an important observation is the approximately 2-fold decrease in MAO-A inhibition activity of 3a compared to the sulphur analogue 2a. Interestingly, inhibition activity increases with various substituents such as for compounds 3b, 3d and 3e with IC_{50} values in the low micromolar range (1.21–2.03 μM). For the organotellurium compounds 4a-d, a further decrease in MAO-A inhibition potency is observed. Indeed, derivative 4a was 5-fold weaker in potency compared to the sulphur analogue 2a, and 2-fold weaker than the selenium analogue 3a. The addition of substituents to derivative 4a increases the MAO-A inhibition potency (e.g., 4b and 4c) but bulkier groups such as the trimethoxyphenyl moiety of 4d resulted in the complete loss of inhibition of both MAO isoforms. For the inhibition of MAO-B, the selenium derivatives 3a-g were the most potent inhibitors. A replacement of selenium with tellurium drastically decreased the inhibition potency with 4a, 4b and 4d displaying no inhibition at 100 μM. A reduction in activity is also observed when selenium is replaced with sulphur (e.g., 2a-d), which shows that this element essential for activity against MAO-B.

4. *IN VIVO* studies

4.1. *2a and 3a strengthen long-term recognition memory consolidation*

To investigate the impact of compounds 2a and 3a on recognition memory we used two behavioural paradigms: the novel object recognition and the social discrimination tests. These tasks rely on the motivational strength of novelty, being purely based in the natural tendency of rodents to seek out and explore novelty, either a novel object or a novel juvenile. The experimental protocols used in these paradigms were also similar: in both cases the animals were habituated to the arena 24 h prior to the acquisition session in which they were presented to the stimuli (two similar objects or one juvenile congener). The tested compounds were given immediately after this session to investigate their impact on memory consolidation. Retention tests were performed after

Table 2
Inhibition of recombinant human MAO-A and MAO-B by compounds 2–4.

Cmp	MAO-A IC$_{50}$ ± SD (μM)	MAO-B IC$_{50}$ ± SD (μM)
2a	4.72 ± 0.221	57.3 ± 19.7
2b	3.89 ± 0.297	No inhibition
2c	0.724 ± 0.042	64.8 ± 11.3
2d	7.82 ± 0.566	No inhibition
3a	9.79 ± 2.57	No inhibition
3b	2.03 ± 0.108	36.5 ± 9.58
3c	5.17 ± 0.207	16.7 ± 2.55
3d	1.68 ± 0.223	2.79 ± 0.142
3e	1.21 ± 0.084	36.8 ± 8.10
3f	10.5 ± 0.783	13.4 ± 2.38
3g	7.15 ± 0.512	21.3 ± 2.69
4a	25.4 ± 0.566	No inhibition
4b	6.66 ± 0.825	No inhibition
4c	14.0 ± 1.19	84.2 ± 13.8
4d	No inhibition	No inhibition
Harmine	0.0041 ± 0.00007	–
Isatin	8.43 ± 0.245	3.90 ± 0.792

24 or 48 h, when the subject mouse was presented to a novel object or a novel social stimulus along with those encountered in the previous session. The inter-trial intervals were chosen based on our previous experiences, since at this time-points the control animals are normally unable to discriminate between novel and familiar stimuli [27–29]. This experimental design represents an advantage when investigating the intrinsic memory-promoting potential of new compounds because exploiting natural forgetting avoids the use of amnesic drugs, thus ruling out issues related to possible pharmacokinetic interactions [30].

The results obtained in the NOR are depicted in Fig. 2. As expected, vehicle-treated mice were unable to remember the previously encountered object, spending a similar percentage of time exploring the familiar and the novel objects (Fig. 2B). **D-Phe** was used as comparator.

Mice receiving the lower doses (25 nmol/μL) of either compound behaved as control mice treated with vehicle. On the contrary, mice treated with the higher doses (50 nmol/μL) remembered the familiar object, hence they spent significantly more time exploring the new object ($p < 0.001$, Fig. 2B). Similar effects were observed following the administration of **D-Phe**: when infused at higher concentration, it elicited a procognitive effect ($p < 0.0001$, Fig. 2B) whereas it was ineffective when given at the lower concentration. Statistically significant

differences between groups were also observed in the discrimination indexes (DI) (Fig. 2C). The DIs calculated for the groups of animals receiving infusions of compounds at the higher concentration were significantly greater (**2a**: 0.185 ± 0.178, $p < 0.05$; **3a**: 0.206 ± 0.241 $p < 0.01$; **D-Phe**: 0.375 ± 0.144 $p < 0.0001$) than the DI calculated for the vehicle-treated group (-0.106 ± 0.114). Conversely, no differences emerged among the DIs determined for the control group and for the groups treated with the lowest dose of each tested compound (**2a**: 0.111 ± 0.271; **3a**: $0.006- \pm 0.177$; **D-Phe**: 0.075 ± 0.190).

In the second set of experiments, we investigated the impact of the same compounds on the consolidation of a long-term social recognition memory. The results obtained (shown in Fig. 3) are largely comparable to those observed in the NOR.

During the retention test, control animals treated with vehicle failed to discriminate between novel and familiar juveniles, spending an equal percentage of time exploring both stimuli. The same behaviour was observed for the animals treated with **2a**, **3a** or **D-Phe** at a dose of 25 nmol/μL. Instead, the animals receiving infusions of either compound at a dose of 50 nmol/μL showed a clear preference towards the social novelty, spending a significantly longer time exploring the novel juvenile with respect to the one met during the acquisition phase (Fig. 3B). In
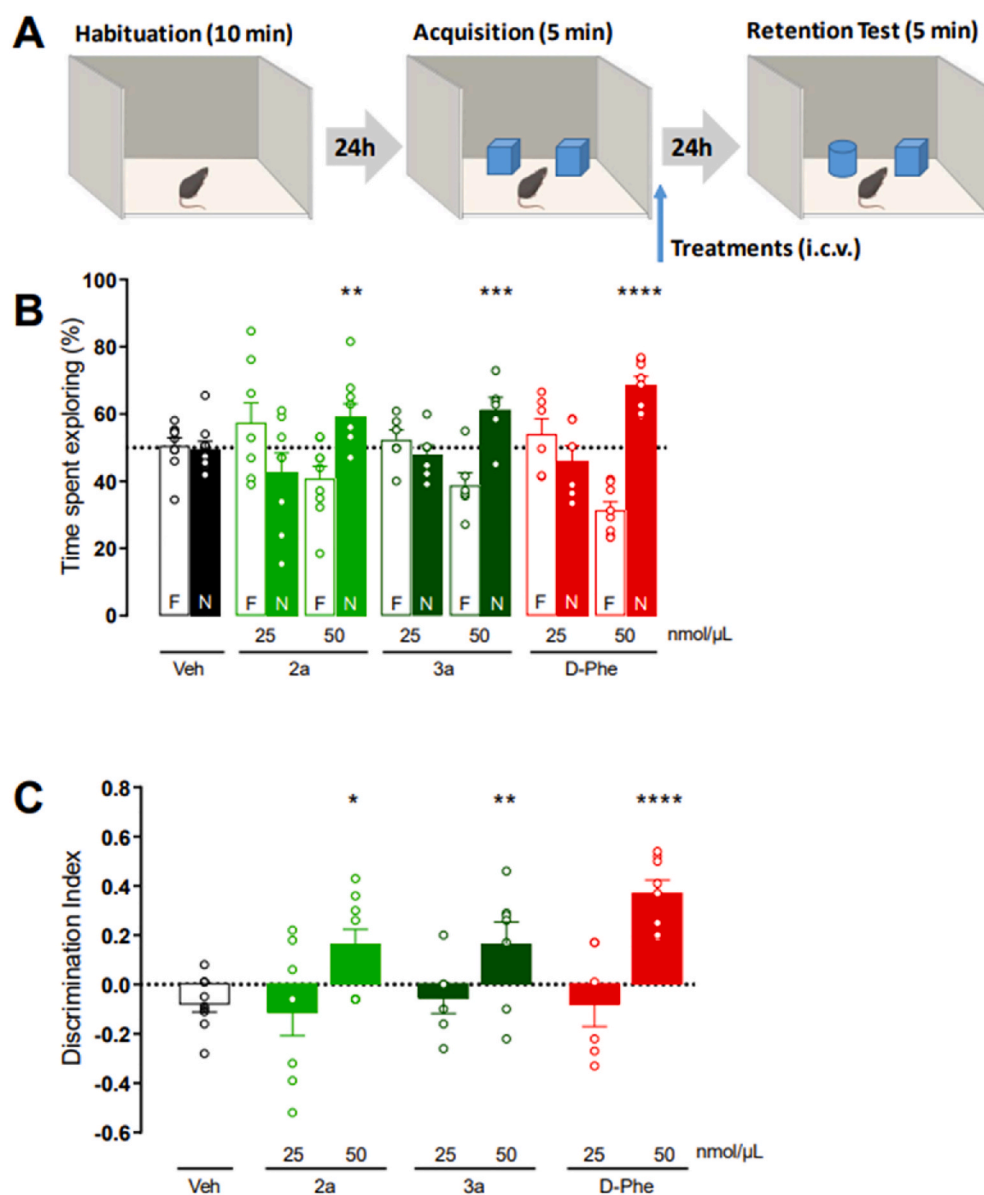

Fig. 2. β-arylchalcogeno amines improve the consolidation of the long-term object recognition memory. (A) Schematic drawing showing the sequence of the behavioural procedures: immediately after the acquisition phase mice received infusions of either vehicle, **2a**, **3a** or **D-Phe** at 25 or 50 nmol/μL. The retention test was performed 24 h later. Data are expressed as individual data points for each animal and bars represent means ± SEM. (B) Percentages of time spent exploring were analyzed by two-way ANOVA followed by Bonferroni's Multiple Comparison Test. Dashed line indicates the theoretical means of 50%. **$P < 0.01$; ***$P < 0.001$ ****$P < 0.0001$ familiar *vs.* novel object within the same group. (C) Discrimination indexes were analyzed by one-way ANOVA followed by Bonferroni's Multiple Comparison Test *$P < 0.05$; **$P < 0.001$ ****$P < 0.0001$ *vs.* vehicle group.
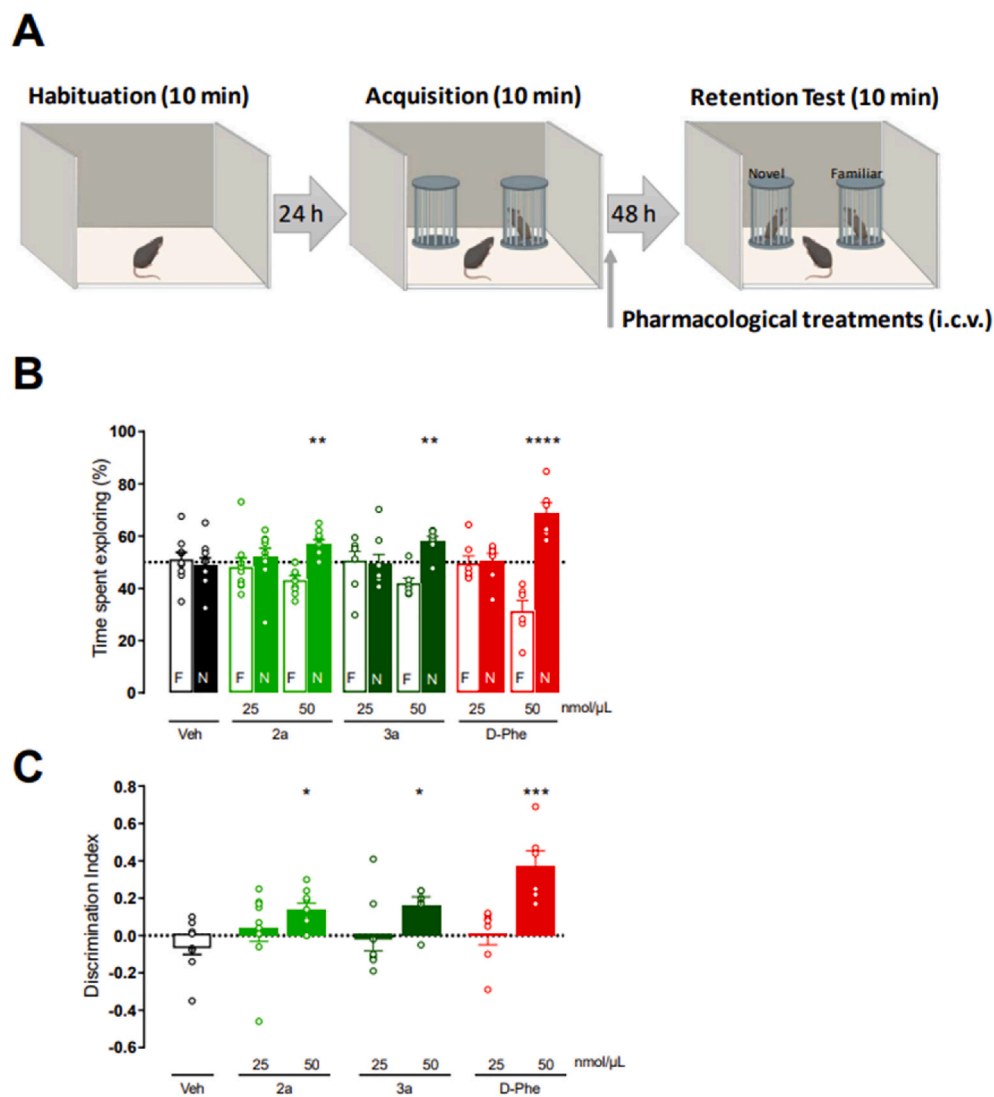
Fig. 3. β-arylchalcogeno amines improve the consolidation of the long-term social recognition memory. (A) Schematic drawing showing the sequence of the behavioural procedures: immediately after the acquisition phase mice received infusions of either vehicle, **2a**, **3a** or **D-Phe** at 25 or 50 nmol/μL. The retention test was performed 48 h later. Data are expressed as individual data points for each animal and bars represent means ± SEM. (B) Percentages of time spent exploring were analyzed by two-way ANOVA followed by Bonferroni's Multiple Comparison Test. Dashed line indicates the theoretical means of 50%. **P < 0.01; ****P < 0.0001 familiar *vs.* novel social stimulus within the same group. (C) Discrimination indexes were analyzed by one-way ANOVA followed by Bonferroni's Multiple Comparison Test *P < 0.05; ***P < 0.0001 *vs.* vehicle group.

agreement with these results, the DIs of mice treated with the compounds at the lower dose (**2a**: 0.038 ± 0.210; **3a**: 0.011 ± 0.202; **D-Phe**: 0.009 ± 0.151) did not differ from the controls values (-0.078 ± 0.121), whereas the DIs of the animals receiving the highest dose significantly increased (**2a**: 0.138 ± 0.104, $p < 0.05$; **3a**: 0.156 ± 0.101 $p < 0.05$; **D-Phe**: 0.291 ± 0.142 $p < 0.001$) (Fig. 3C).

4.2. 2a and 3a did not elicit AMP-like behaviours

AMP and AMP-like compounds are a class of drugs known to induce significant stimulant effects in the brain, suppressing appetite and promoting hyperkinesia [31]. In view of the structural similarities between AMP and **2a** and **3a**, we decided to evaluate the effects of these compounds on motor activity and food consumption and compare them with the amphetamine-elicited effects. The dose of amphetamine used in our study is similar to that used in other studies [32] and caused a substantial reduction in food intake as well as significant locomotor activation.

The distance covered during the 5-min session by the mice treated with **2a**, **3a** or **AMP** at 50 nmol/μL is presented in Fig. 4.

As expected, **AMP** treatment elicited a significant increase in locomotor activity as revealed by the significantly longer distances covered by the amphetamine-treated mice with respect to the animals receiving vehicle infusions ($p < 0.01$). On the other hand, no differences in terms

of distance travelled were observed for the groups of animals treated with either **2a** or **3a** with respect to the control group (Fig. 4B).

The effects of the different compounds on food intake were assessed using the fasting-refeeding protocol. **AMP** infusion caused a profound reduction of food consumption ($p < 0.05$). The onset of the **AMP** hypophagic effect was fast as it was already significant 30 min after food presentation and persisted for the 2 h evaluating time. Conversely animals treated with either **2a** or **3a** consumed comparable amounts of food with respect to control animals (Fig. 5).

4.3. Effects of compound 2a and 3a systemic treatment on brain CA activity

In order to examine the influences of compounds **2a** and **3a** systemic treatment on brain CA activity, mice were euthanized 30 min after the i. c.v. infusion, their brains were collected, and the hippocampi and cortices were immediately dissected on ice and then homogenized individually in 1 mL of ice cold 20 mM Hepes buffer (pH 7.5). Controls received a comparable injection of vehicle and their brains were collected and processed are treated as mentioned above. The overall catalytic activity of CAs (K_{cat}) of each individual preparation was measured with a stopped flow spectrophotometric method monitoring the physiological reaction catalyzed by these enzymes and the results are shown in Fig. 6. One-way ANOVA revealed an overall difference in
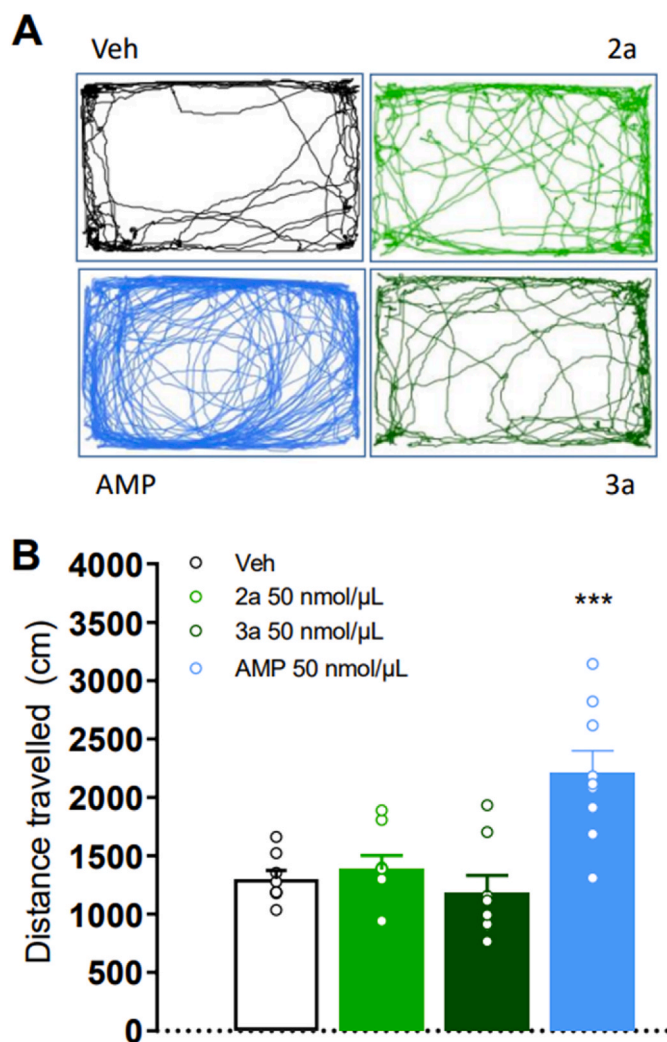
Fig. 4. Amphetamine-like hyperlocomotion was not observed following β-arylchalcogeno amines administration. (A) Representative track plot reports (ANY-maze®) recorded during 5-min Open Field test sessions performed 30 min after administration of vehicle, **2a**, **3a** or **AMP** at 50 nmol/μL directly into the lateral ventricles. (B) Quantification of the distance travelled during 5-min Open Field test sessions. Data are expressed as individual data points for each animal and bars represent means ± SEM. Data was analyzed with one-way ANOVA followed by Bonferroni's Multiple Comparison, ***P < 0.001 *vs*. vehicle group.

activity between the control group and the treated groups, in particular the group treated with compound **3a** (P < 0.0001) showing that brain mean activity was significantly different from that of saline-treated control animals.

Lastly, keeping into consideration that lipophilic amine pharmacophore was known to be promiscuous, we explored the structures of compounds **2a** and **3a** by means of SwissADME and Swisstargetprediction webtools [33]. For both compounds, the former displayed high gastrointestinal absorption and Blood Brain Barrier permeation, limited ability to behave as a substrate to P-glycoprotein and the possibility to be metabolised by CYP1A2 (only for compound **2a**). In addition, the substitution of the sulphur atom (consensus Log $P_{w/o}$ 2.23) with a selenium (consensus Log $P_{w/o}$ 1.13) led to an improvement of hydrophilicity as calculated by the software. This could allow the administration not only by our selected i.c.v. route, but also orally in order to obtain satisfactory concentrations in the brain practical for a cognition therapeutic. Moreover, both of them respected the Lipinski's rule and cannot be considered PAINS, thus demonstrating the
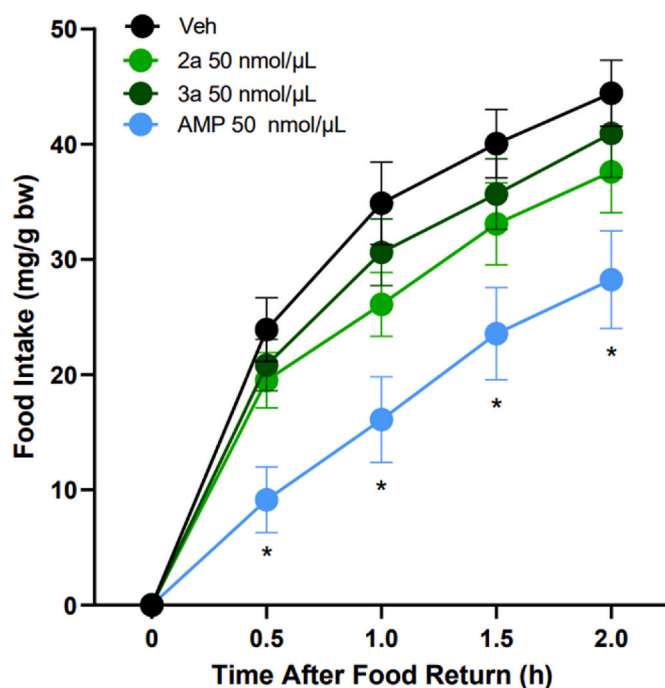

Fig. 5. AMP-like appetite suppression was not observed following β-arylchalcogeno amines administration. Overnight fasted animals were treated with vehicle, **2a**, **3a** or **AMP** at 50 nmol/μL directly into the lateral ventricles. Thirty min later, the food was presented and the consumption was recorded in the following 2 h at 0.5 h intervals. Cumulative food consumption was corrected considering individual body weight. Data are expressed as individual means ± SEM. Data was analyzed with repeated measures two-way ANOVA followed by Bonferroni's Multiple Comparison, *P < 0.001 *vs*. vehicle group (n = 8–9 mice in each experimental group).

rationale behind our choice to further assay them in *in vivo* experiments. The latter suggested that this scaffold had few off-targets which it can interact with, because the results indicated a low probability score towards melatonin receptors and sodium channels and no results for dopamine β-hydroxylase. Interestingly, the webtool also suggested a role for FAD-containing amine oxidase as we performed.

5. Conclusion

In summary, we reported the synthesis of β-arylchalcogeno amines (**2–4**) as structural analogues of amphetamine and, evaluated the efficacy of two compounds (**2a** and **3a**) to counteract time-induced cognitive impairments in mice models of recognition memory, without inducing hyperlocomotion and hypophagia, two classical AMP-like effects, following their administration. From the general point of view, CA activation by the designed compounds was more potent for hCA IV and hCA XIV, two of the most abundant isoforms present in cortical and hippocampal brain structures and good potency of activation, as well as for hCA VII, one of the most widely expressed isoforms in the brain which is involved in oxidative stress. Moreover, these compounds showed selective MAO-A inhibition profile, which, together with the antioxidant activity reported earlier [19], reduces overall oxidative stress. Taken together, these data showed a distinct pharmacological profile of our β-arylchalcogeno amines, which could be employed as innovative tools to treat cognitive impairments observed in pathologies such as attention deficit disorder, Parkinson's disease-related cognitive dysfunction and cognitive disorders associated with depression.
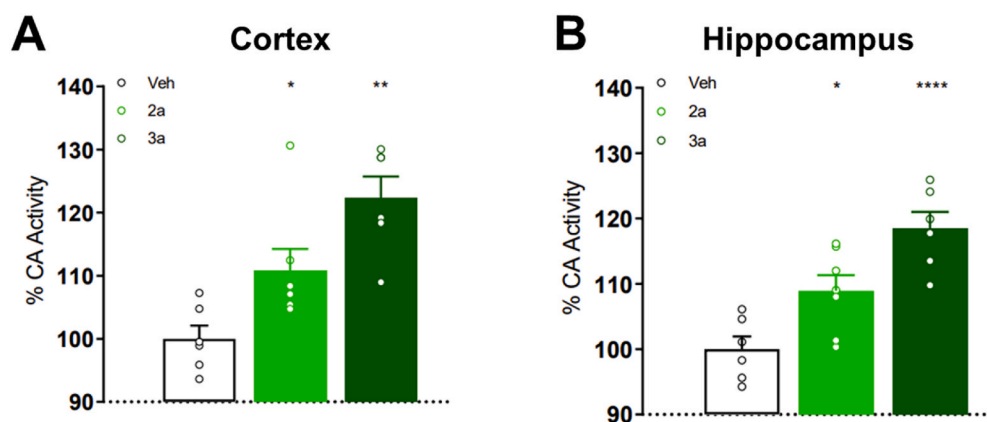
Fig. 6. CA activity (%) in cortical (A) and hippocampal (B) brain homogenates obtained from mice treated with vehicle or compounds **2a** and **3a** (50 nmol/μL, i.c.v.). Animals were sacrificed 30 min after drug infusion and the brain structures immediately dissected on ice and homogenized. CA activity was measured with a stopped flow method. The CA mean activity of control group mice was taken as 100%. Data are expressed as means ± S.E.M. of 6 mice per experimental group. *P < 0.05 **P < 0.01, ****P < 0.0001 *vs.* vehicle (One-way ANOVA).

6. Experimental section

6.1. General

Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, VWR and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR, ^{77}Se NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; bs, broad singlet; dd, doublet of doublets. The assignment of exchangeable protons (N*H*) was confirmed by the addition of D$_2$O. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck silica gel 60 (230–400 mesh ASTM) as the stationary phase, and ethyl acetate, *n*-hexane, acetonitrile and methanol were used as eluents. The solvents used in MS measurements were acetone, acetonitrile (Chromasolv grade), purchased from Sigma-Aldrich (Milan, Italy), and mQ water (18 MΩ), obtained from Millipore's Simplicity system (Milan, Italy). Mass spectra were obtained using a Varian 1200L triple quadrupole system (Palo Alto, CA, USA) equipped with electrospray source (ESI) operating in both positive and negative ions. Stock solutions of analytes were prepared in acetone at 1.0 mg mL^{-1} and stored at 4 °C. Working solutions of each analyte were freshly prepared by diluting stock solutions in a mixture of mQ H$_2$O/ACN 1/1 (*v/v*) up to a concentration of 1.0 μg mL^{-1} The mass spectra of each analyte were acquired by introducing, *via* syringe pump at 10/L min^{-1}, the working solution. Raw data were collected and processed by Varian Workstation, version 6.8, software. All compounds reported here are >95% of purity by NMR.

6.2. Chemistry

Characterisation of compounds 2a-d, 3a-g and 4a-d were reported earlier by our group [22,23].

6.3. Carbonic anhydrase assay

A stopped-flow method [24] was used for assaying the CA catalysed CO$_2$ hydration activity with phenol red as indicator, working at the absorbance maximum of 557 nm, following the initial rates of the CA-catalysed CO$_2$ hydration reaction for 10–100 s. For each activator at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of activator (0.1 mM) were prepared in distilled–deionised

water and dilutions up to 0.1 nM were done thereafter with the assay buffer. The activation constant (K_A), defined similarly to the inhibition constant K_I, was obtained by considering the classical Michaelis–Menten equation (Equation (1)), which has been fitted by non-linear least squares by using PRISM 3:

$$V = V_{max} / \{1 + K_M/[S] (1 + [A]_f / K_A)\} \quad (1)$$

where $[A]_f$ is the free concentration of activator. Working at substrate concentrations considerably lower than K_M ($[S] \ll K_M$), and considering that $[A]_f$ can be represented in the form of the total concentration of the enzyme ($[E]_t$) and activator ($[A]_t$), the obtained competitive steady-state equation for determining the activation constant is given by Equation (2) [34–37]:

$$V = V_0 K_A / \{K_A + ([A]_t - 0.5\{([A]_t + [E]_t + K_A) - ([A]_t + [E]_t + K_A)^2 - 4 [A]_t[E]_t)^{1/2} \}\} \quad (2)$$

where V_0 represents the initial velocity of the enzyme-catalyzed reaction in the absence of activator. All CA isozymes used in the experiments were purified recombinant proteins obtained as reported earlier by our group [34–37].

6.4. MAO inhibition assays

The catalytic activities of MAO-A and MAO-B were measured as reported in literature [26,38]. Recombinant human MAO-A and MAO-B are commercially available (Sigma-Aldrich) and served as enzyme sources. Kynuramine was used as MAO-A/B non-specific substrate and is oxidized by the MAOs to ultimately yield 4-hydroxyquinoline, which was measured by fluorescence spectrophotometry. The *in vitro* measurement of MAO activity was approved by the North-West University Animal Care, Health and Safety Research Ethics Committee (NWU-AnimCareREC) with the following ethics number: NWU-00572-19-A5 (A. and J. Petzer).

6.5. In vivo assay

6.5.1. Compounds
Compounds 2a and 3a were prepared and characterized as described above. D-Phe was purchased from Sigma-Aldrich. AMP was provided by Dr. Fabio Vaiano (University of Florence). All compounds were dissolved in physiological saline containing DMSO 1% (v/v) to obtain solutions with final concentration of 25 and 50 nmol/μL. The solutions were freshly prepared and infused directly into the lateral ventricles as described below.

6.5.2. Animals
Juvenile (3–4 weeks old) and adult (8–9 weeks old) C57Bl6 male mice were used for behavioural experiments. These animals were bred at

the animal facility of the Centro di Servizi per la Stabulazione di Animali da Laboratorio (CeSAL) of the University of Florence. They were housed in humidity and temperature-controlled rooms (22 $\pm$ 2 °C), allowed free access to food (4RF21; Mucedola s.r.l., Italy) and water, and kept on a 12-h light/dark cycle (lights start at 8:00 a.m.). Breeding, housing and all the experimental procedures were conducted in accordance with the Council Directive of the European Community (2010/63/EU) and the Italian Decreto Legislativo 26 (March 13, 2014), approved by the Animal Care Committee of the University of Florence and Italian Ministry of

subjected to the procedure separately and care was taken to remove any olfactory/taste cues by cleaning carefully the arena and test objects between trials with an ehtanolic solution (30% v/v). Mice were placed in their home cages between trials. The final data is expressed as the percentage of time exploring the familiar and new objects during the retention test. The raw exploration time data was employed also to calculate the discrimination index, according to the following equation:

$$Discrimination\ Index\ (DI) = \frac{time\ esploring\ novel\ (tN) - time\ exploring\ familiar\ (tF)}{total\ exploration\ time\ (tN + tF)}$$

Health and supervised by a veterinarian. Every effort was made to minimize animal suffering and to reduce the number of animals used.

Male mice were used to decrease within-group variability due to hormonal fluctuations during estrous cycle in female mice. Animals were handled for at least 4 days before experiments begun, to let them acclimatize to human contact. All the experiments were conducted between 9:00 a.m. and 4:00 p.m.

6.5.3. Stereotaxic surgery and infusion procedure

Mice were anesthetized using a mixture of ketamine (15 mg/kg) and xylazine (2.5 mg/kg) and placed in a stereotaxic frame equipped with a mouse adapter and ear bars (Stoelting, Chicago, USA). A stainless steel cannula (7 mm in length, outer diameter 0.5 mm, and inner diameter 0.25 mm) was then implanted in the lateral ventricle and fixed to the skull using dental cement. The following coordinates were used according to the mouse brain atlas [39] antero-posterior (AP) −0.3 mm; lateral (L) + 1 mm; dorsoventral (DV) −1 mm. Animals were then left to recover for a period of 7 days. In the infusion day, a stainless-steel injection micro-needle (outer diameter 0.25 mm) was connected through a polyethylene catheter to a 1000 μL Hamilton precision syringe and then lowered into the lateral cerebral ventricle (DV 2.4 mm). Five μL of a solution containing the different drugs were delivered using an infusion pump at 1 μL/min flow. Animals in the control group received equal volumes of vehicle. The needle was left in place for 1 additional min after each infusion [29,40].

6.5.4. Novel object recognition test

One week after the stereotaxic surgery, mice behaviour was assessed in an open-field arena (60 × 70 cm and 30 cm high) constructed in white acrylic Plexiglass and placed in a sound attenuated room. The detailed experimental procedure was previously published [27,28]. In brief, the protocol involved a total of three sessions: habituation, acquisition and retention test separated by 24 h inter-trial intervals. During habituation, the animals freely explored the empty arena for 10 min. Twenty-four hours later, each animal was placed in the same position and facing the same direction into the test arena in the presence of two identical objects (grey plastic shapes such as cubes, cylinders or pyramids 8 cm high) and left to explore it for 5 min. Immediately after this session the animals received microinfusions of the different treatments directly into the lateral ventricles. The retention test session was performed 24 h after acquisition, during which, each mouse was again placed in the test arena for 5 min in the presence of one of the familiar object and a novel object. The position of the new objects (left/right) was randomized to prevent bias from order or place preference. Animal behaviour during all session was videotaped and the time spent actively exploring each object was recorded by an experienced observer unaware of the experimental groups. Exploration was defined as sniffing or touching the stimulus object with the nose and/or forepaws. Sitting on or turning around the objects was not considered exploratory behaviour. Each animal was

6.5.5. Social discrimination paradigm

Social recognition memory was evaluated using the social discrimination paradigm as previously described [29]. The experimental apparatus used was an open-field Plexiglas arena (45 × 25 cm and 20 cm high) placed in a sound attenuated room. Two identical wire pencil cups (9 cm diameter × 13 cm high) were positioned inside the arena on opposing sides. The wire material allows the passage of olfactory cues while preventing direct interaction between adults and juveniles. All juvenile stimulus stimulus mice were habituated to remain under the wire cups for 30 min during several days before behavioural testing. The experimental protocol comprises three sessions: habituation, acquisition and retention test. During the habituation, adult animals were left free to explore the arena containing two empty cups for 10 min. Twenty-four hours later, during acquisition, a juvenile mouse (stimulus, 3–4 weeks old), which had no prior contact with the subject mice, was placed under one of the wire cups while the other cup remained empty. The subject mouse was then placed in the arena and was left free to explore it for 10 min. At the end of this session, the drugs were infused directly into the lateral ventricles. The retention test occurred 48 h later, in which the adult animals were placed again in the arena containing the previously presented juvenile (familiar) and a novel one placed under the cup that had been empty during the sample phase. This session lasted 10 min. The two juvenile stimuli came from different home cages to prevent the redundancy of olfactory cues and the second juvenile had no prior contact with the adult animal. The arena and the cups were cleaned with ethanol (30% v/v) between trial to remove any olfactory/taste cues. The position of the social stimuli (empty × social; familiar × novel) were counterbalanced across subjects and trials to prevent bias due to place preference. All the animals returned to their home cages between sessions.

The animal's behaviour during all sessions was videotaped, and the time spent actively exploring the stimuli was analyzed by experienced observers blinded to the pharmacological treatments. Exploration was defined as direct snout-to-cup contact, and the time spent climbing on the cups was not considered. Data are expressed as a percentage of time spent exploring each cup (familiar × novel) during the retention test session. A discrimination index (DI) was also calculated according to the formula described above.

6.5.6. Open field

Locomotor activity was assessed in an open field arena (60 × 70 cm and 30 cm high) constructed in white acrylic Plexiglass using the protocol described in Ref. [27]. In brief, one week after the surgeries, the animals received i.c.v. infusions of the solutions containing either vehicle or the compound to be tested at the highest effective dose. Thirty minutes later they were placed in the arena and allowed to freely explore it for 5 min. The arena was cleaned with a solution of ethanol 30% (v/v) to remove possible scent cues left by the animal between trials. A camera located on the ceiling above the apparatus recorded the experiments.

The total distance travelled was recorded and analyzed using the Any-Maze® (version 7.13) video tracking system.

6.5.7. Evaluation of food consumption

Cumulative food consumption was evaluated using the fasting-refeeding protocol [41]. Single housed mice were submitted to an overnight food deprivation while water remained available ad libitum. In the next morning (9:00 a.m.), they received the different treatments directly into the lateral ventricle trough the cannulas previously implanted. Thirty minutes later, a weighed amount of standard chow pellets was placed in their food rack. Food consumption was evaluated 0.5, 1, 1.5 and 2 h after food presentation measuring the difference in weight between that of initially provided food and that left in the rack, including spillage in the cage. The amount of food consumed was corrected by the body weight of each animal and expressed as mg/g body weight.

6.6. Ex vivo measurement of CA activity in brain samples

Mice were randomly divided in three groups and received infusions directly into the lateral ventricles, through a cannula previously implanted, of 5 μL of solutions containing vehicle, 2a (50 nmol/μL) or 3a (50 nmol/μL). Thirty minutes after drug injections, they were euthanized, the brain immediately removed and their cortices and hippocampi were rapidly dissected on ice, weighted, and stored at -80 °C until use. For determination of CA activity, brain tissues were homogenized in 1 mL of ice-cold 20 mM Hepes buffer (pH 7.5). After centrifugation at 20000g for 30 min, an Applied Photophysics stopped-flow instrument was used for assaying the CA-catalyzed CO_2 hydration activity from the samples as previously described [12]. Briefly, phenol red (at a concentration of 0.2 mM) was used as an indicator working at the absorbance maximum of 557 nm, with 20 mM Hepes buffer (pH 7.5) and 20 mM $NaClO_4$ for maintaining constant ionic strength, following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s, at 20 °C. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and activation constants. The mean CA activity determined in the brain samples from mice treated with vehicle was taken as 100% and all the individual calculated values were then expressed as a percentage of controls [12].

6.7. Statistical analysis

Behavioural experimental data are expressed as individual points for each animal (scattered plot). The bar graph represent means ± standard error of the mean (S.E.M). These data were analyzed using Graphpad Software (version 9.0). The percentage of time the animals spent exploring the different stimuli and the cumulative food consumption were analyzed with two-way ANOVA. The discrimination indexes and the distance travelled in the open field were analyzed with One-way ANOVA. The source of the detected significances was determined by Bonferroni's multiple comparison post-hoc test. P values less than 0.05 were considered statistically significant. The number of mice per experimental group is indicated in their respective figure legends.

Authors contributions

The enzymology involving MAO was carried out by AP and JPP at the North-West University. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Founding source

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Abbreviations

CA	carbonic anhydrase
MAO	monoamine oxidase
AMP	amphetamine
D	Phe
D	phenylalanine
CAA	carbonic anhydrase activator

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2022.114828.

References

[1] P.D. Harvey, Domains of cognition and their assessment Dialogues, Clin. Neurosci. 21 (2019) 227–237.

[2] J.E. Morley, J.C. Morris, M. Berg-Weger, S. Borson, B.D. Carpenter, N. Del Campo, B. Dubois, K. Fargo, L.J. Fitten, J.H. Flaherty, M. Ganguli, G.T. Grossberg, T. K. Malmstrom, R.D. Petersen, C. Rodriguez, A.J. Saykin, P. Scheltens, E. G. Tangalos, J. Verghese, G. Wilcock, B. Winblad, J. Woo, B. Vellas, Brain health: the importance of recognizing cognitive impairment: an IAGG consensus conference, J. Am. Med. Dir. Assoc. 16 (2015) 731–739.

[3] A. Morozova, Y. Zorkina, O. Abramova, O. Pavlova, K. Pavlov, K. Soloveva, M. Volkova, P. Alekseeva, A. Andryshchenko, G. Kostyuk, O. Gurina, V. Chekhonin, Neurobiological highlights of cognitive impairment in psychiatric disorders, Int. J. Mol. Sci. 23 (2022) 1217.

[4] A. Etkin, A. Gyurak, R.A. O'Hara, Neurobiological approach to the cognitive deficits of psychiatric disorders, Dialogues Clin. Neurosci. 15 (2013) 419–429.

[5] T. Qarni, A. Salardini, A multifactor Approach to mild cognitive impairment, Semin. Neurol. 39 (2019) 179–187.

[6] L.M. McTeague, J. Huemer, D.M. Carreon, Y. Jiang, S.B. Eickhoff, A. Etkin, Identification of common neural circuit disruptions in cognitive control across psychiatric disorders, Am. J. Psychiatr. 174 (2017) 676–685.

[7] P. Blandina, G. Provensi, M.B. Passsani, C. Capasso, C.T. Supuran, Carbonic anhydrase modulation of emotional memory. Implications for the treatment of cognitive disorders, J. Enzym. Inhib. Med. Chem. 35 (2020) 1206–1214.

[8] V. Poggetti, S. Salerno, E. Baglini, E. Barresi, F. Da Settimo, S. Taliani, Carbonic anhydrase activators for neurodegeneration: an overview, Molecules 27 (2022) 2544.

[9] M.K. Sun, D.L. Alkon, Pharmacological enhancement of synaptic efficacy, spatial learning, and memory through carbonic anhydrase activation in rats, J. Pharmacol. Exp. Therapeut. 297 (2001) 961–967.

[10] P.W. Pan, A.K. Parkkila, S. Autio, M. Hilvo, R. Sormunen, S. Pastorekova, J. Pastorek, H. Haapasalo, S. Parkkila, Brain phenotype of carbonic anhydrase IX-deficient mice, Transgenic Res. 21 (2012) 163–176.

[11] M.T. Yang, W.L. Chien, D.H. Lu, H.C. Liou, W.M. Fu, Acetazolamide impairs fear memory consolidation in rodents, Neuropharmacology 67 (2013) 412–418.

[12] L. Canto de Souza, G. Provensi, D. Vullo, F. Carta, A. Scozzafava, A. Costa, S. D. Schmidt, M.B. Passani, C.T. Supuran, P. Blandina, Carbonic anhydrase activation enhances object recognition memory in mice through phosphorylation of the extracellular signal-regulated kinase in the cortex and the hippocampus, Neuropharmacology 118 (2017) 148–156.

[13] S.D. Schmidt, A. Costa, B. Rani, E. Godfried Nachtigall, M.B. Passani, F. Carta, A. Nocentini, I. de Carvalho Myskiw, C.R.G. Furini, C.T. Supuran, I. Izquierdo, P. Blandina, G. Provensi, The role of carbonic anhydrases in extinction of contextual fear memory, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 16000–16008.

[14] S.D. Schmidt, E.G. Nachtigall, L.A. Marcondes, A. Zanluchi, C.R.G. Furini, M. B. Passani, C.T. Supuran, P. Blandina, I. Izquierdo, G. Provensi, J. de Carvalho Myskiw, Modulation of carbonic anhydrases activity in the Hippocampus or prefrontal cortex differentially affects social recognition memory in rats, Neuroscience S0306–4522 (22) (2022), 00148-8.

[15] A. Di Fiore, D.M. Monti, A. Scaloni, G. De Simone, S.M. Monti, Protective role of carbonic anhydrases III and VII in cellular defense mechanisms upon redox unbalance, Oxid. Med. Cell. Longev. (2018), 2018306, 2018.

[16] X.Y. Zhang, J.K. Yao, Oxidative stress and therapeutic implications in psychiatric disorders, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 46 (2013) 197–199.

[17] C.C. Wang, E. Billett, A. Borchert, H. Kuhn, C. Ufer, Monoamine oxidases in development, Cell. Mol. Life Sci. 70 (2013) 599–630.

[18] T. Behl, D. Kaur, A. Sehgal, S. Singh, N. Sharma, G. Zengin, F.L. Andronie-Cioara, M.M. Toma, S. Bungau, A.G. Bumbu, Role of monoamine oxidase activity in alzheimer's disease: an insight into the therapeutic potential of inhibitors, Molecules 26 (2021) 3724.

[19] A. Angeli, F. Vaiano, F. Mari, E. Bertol, C.T. Supuran, Psychoactive substances belonging to the amphetamine class potently activate brain carbonic anhydrase isoforms VA, VB, VII, and XII, J. Enzym. Inhib. Med. Chem. 32 (2017) 1253–1259.

[20] M. Reyes-Parada, P. Iturriaga-Vasquez, B.K. Cassels, Amphetamine derivatives as monoamine oxidase inhibitors, Front. Pharmacol. 10 (2020) 1590.

[21] M. Naoi, P. Riederer, W. Maruyama, Modulation of monoamine oxidase (MAO) expression in neuropsychiatric disorders: genetic and environmental factors involved in type A MAO expression, J. Neural. Transm. 123 (2016) 91–106.

[22] D. Tanini, C. Borgogni, A. Capperucci, Mild and selective silicon-mediated access to enantioenriched 1,2-mercaptoamines and β-amino arylchalcogenides, New J. Chem. 43 (2019) 6388–6393.

[23] D. Tanini, A. Capperucci, C.T. Supuran, A. Angeli, Sulfur, selenium and tellurium containing amines act as effective carbonic anhydrase activators, Bioorg. Chem. 87 (2019) 516–522.

[24] R.G. Khalifah, The carbon dioxide hydration activity of carbonic anhydrase. I. Stop flow kinetic studies on the native human isoenzymes B and C, J. Biol. Chem. 246 (1971) 2561–2573.

[25] L. Novaroli, M. Reist, E. Favre, A. Carotti, M. Catto, P.A. Carrupt, Human recombinant monoamine oxidase B as reliable and efficient enzyme source for inhibitor screening, Bioorg. Med. Chem. 13 (2005) 6212–6217.

[26] H. Weissbach, T.E. Smith, J.W. Daly, B. Witkop, S. Udenfriend, A rapid spectrophotometric assay of mono-amine oxidase based on the rate of disappearance of kynuramine, J. Biol. Chem. 235 (1960) 1160–1163.

[27] A. Costa, B. Rani, T.F.S. Bastiaanssen, F. Bonfiglio, E. Gunnigle, G. Provensi, M. Rossitto, M. Boehme, C. Strain, C.S. Martínez, P. Blandina, J.F. Cryan, S. Layé, R. Corradetti, M.B. Passani, Diet prevents social stress-induced maladaptive neurobehavioural and gut microbiota changes in a histamine-dependent manner, Int. J. Mol. Sci. 23 (2022) 862.

[28] G. Provensi, A. Costa, M.B. Passani, P. Blandina, Donepezil, an acetylcholine esterase inhibitor, and ABT-239, a histamine H3 receptor antagonist/inverse agonist, require the integrity of brain histamine system to exert biochemical and procognitive effects in the mouse, Neuropharmacology 109 (2016) 139–147.

[29] B. Rani, B. Silva-Marques, R. Leurs, M.B. Passani, P. Blandina, G. Provensi, Short- and long-term social recognition memory are differentially modulated by neuronal histamine, Biomolecules 11 (2021) 555.

[30] B. Grayson, M. Leger, C. Piercy, L. Adamson, M. Harte, J.C. Neill, Assessment of disease-related cognitive impairments using the novel object recognition (NOR) task in rodents, Behav. Brain Res. 285 (2015) 176–193.

[31] D.J. Heal, S.L. Smith, J. Gosden, D.J. Nutt, Amphetamine, past and present–a pharmacological and clinical perspective, J. Psychopharmacol. 27 (2013) 479–496.

[32] I. Mahú, A. Barateiro, E. Rial-Pensado, N. Martinéz-Sánchez, S.H. Vaz, P.M.S. D. Cal, B. Jenkins, T. Rodrigues, C. Cordeiro, M.F. Costa, R. Mendes, E. Seixas, M. M.A. Pereira, N. Kubasova, V. Gres, I. Morris, C. Temporão, M. Olivares, Y. Sanz, A. Koulman, F. Corzana, A.M. Sebastião, M. López, G.J.L. Bernardes, A. I. Domingos, Brain-sparing sympathofacilitators mitigate obesity without adverse cardiovascular effects, Cell Metabol. 31 (2020) 1120–1135, e7.

[33] A. Daina, V. Zoete, Application of the SwissDrugDesign online resources in virtual screening, Int. J. Mol. Sci. 20 (2019) 4612.

[34] E. Barresi, R. Ravichandran, L. Germelli, A. Angeli, E. Baglini, S. Salerno, A. M. Marini, B. Costa, E. Da Pozzo, C. Martini, F. Da Settimo, C. Supuran, S. Cosconati, S. Taliani, Carbonic anhydrase activation profile of indole-based derivatives, J. Enzym. Inhib. Med. Chem. 36 (2021) 1783–1797.

[35] A. Angeli, N. Chiaramonte, D. Manetti, M.N. Romanelli, C.T. Supuran, Investigation of piperazines as human carbonic anhydrase I, II, IV and VII activators, J. Enzym. Inhib. Med. Chem. 33 (2018) 303–308.

[36] A. Mollica, G. Macedonio, A. Stefanucci, S. Carradori, A. Akdemir, A. Angeli, C. T. Supuran, Five- and six-membered nitrogen-containing compounds as selective carbonic anhydrase activators, Molecules 22 (2017) 2178.

[37] G. Vistoli, G. Aldini, L. Fumagalli, C. Dallanoce, A. Angeli, C.T. Supuran, Activation effects of carnosine- and histidine-containing dipeptides on human carbonic anhydrases: a comprehensive study, Int. J. Mol. Sci. 21 (2020) 1761.

[38] S. Mostert, A. Petzer, J.P. Petzer, Indanones as high-potency reversible inhibitors of monoamine oxidase, ChemMedChem 10 (2015) 862–873.

[39] K.B.J. Franklin, G. Paxinos, Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates, third ed., Elsevier Academic Press, Amsterdam, Boston, 2019.

[40] L. Munari, G. Provensi, M.B. Passani, N. Galeotti, T. Cassano, F. Benetti, R. Corradetti, P. Blandina, Brain histamine is crucial for selective serotonin reuptake inhibitors' behavioral and neurochemical effects, Int. J. Neuropsychopharmacol. 18 (2015) pyv045.

[41] G. Provensi, R. Coccurello, H. Umehara, L. Munari, G. Giacovazzo, N. Galeotti, D. Nosi, S. Gaetani, A. Romano, A. Moles, P. Blandina, M.B. Passani, Satiety factor oleoylethanolamide recruits the brain histaminergic system to inhibit food intake, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 11527–11532.