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Quinazolinones, Quinazolinthiones, and Quinazolinimines as Nitric Oxide Synthase Inhibitors: Synthetic Study and Biological Evaluation

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The synthesis of different compounds with a quinazolinone, quinazolinthione, or quinazolinimine skeleton and their *in vitro* biological evaluation as inhibitors of inducible and neuronal nitric oxide synthase (iNOS and nNOS) isoforms are described. These derivatives were obtained from substituted 2-aminobenzylamines, using diverse cyclization procedures. Furthermore, the diamines were synthesized by two routes: A conventional pathway and an efficient one-pot synthesis in a continuous-flow hydrogenator. The structures of these heterocycles were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance and high-resolution mass spectroscopy data. The structure–activity relationships of the target molecules are discussed in terms of the effects of both the R radical and the X heteroatom in the 2-position. In general, the assayed compounds behave as better iNOS than nNOS inhibitors, with the quinazolinone **11e** being the most active inhibitor of all tested compounds and the most iNOS/nNOS selective one.

Keywords: Inhibitors / Quinazoline / Synthesis

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Introduction

Nitric oxide synthase (NOS) catalyzes the oxidation of a L-arginine guanidinium nitrogen atom to nitric oxide (NO), a potent biological signaling molecule that mediates a diverse range of physiological processes within the cardiovascular, immune, and nervous systems [1–3]. Three mammalian NOS isoforms have been characterized so far. Two of them, the endothelial (eNOS) and neuronal NOS (nNOS), are constitutive and calcium-dependent. The third isoform, the inducible one (iNOS), is formed in response to pathological challenges [4].

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NO production by nNOS takes part in neuronal signaling, synaptic plasticity, modulation of pathways involved in learning, and neurotoxicity; NO synthesized by eNOS participates in regulation of vascular function, promotes vasodilation, and modulates arterial pressure; and production of NO by iNOS is part of non-specific immunity, it modulates the immune system and acts as proinflammatory mediator [5]. An overproduction of NO by nNOS produces reactive oxygen species (ROS), reactive nitrogen species (RNS), oxidative and nitrosative stress induction, thiole groups nitrosation, phenolic residues nitration, mitochondrial damage, neuronal death, and inflammation. Thus, it has been demonstrated the implication of the nitrergic signaling dysregulation, nNOS overexpression, and oxidative stress in neurodegenerative disorders [6]. Meanwhile, astrocytes and activated glia inflammatory mediators release more NO by iNOS, causing neuronal damage and microglia increased activity [7].

While eNOS-generated NO plays a role in vascular regulation, the potential therapeutic utility of NOS inhibitors is

restricted to the inhibition of the neuronal or inducible isoforms [8]. Overproduction of NO by nNOS is associated with various diseases such as neurodegeneration, stroke, migraine, Alzheimer, and Huntington diseases. On the other hand, overproduction of NO by iNOS is related to hypotensive crises during septic shock, arthritis, colitis, tissue damage, neuropathic pain, and several kinds of inflammatory states [9–12]. Therefore, the use of substances with inhibitory properties of the different NOS isoforms has great therapeutic potential for the treatment of the above-mentioned pathologies [13, 14]. Consequently, over the last 20 years the design and synthesis of NOS inhibitors have received much attention.

The best known inhibitors of NOS with a guanidine moiety are amino acids related to the substrate L-arginine, for example L-NA **1** (or its methyl esther L-NAME, **2**) [15] (Fig. 1). However, none of them is particularly selective. Other inhibitors based on guanidines [16], isothioureas [17, 18], and amidines [19–21] have been reported with different levels of selectivity and potency *in vitro*. Thus, the acetamidine **3** [21] is a potent iNOS inhibitor without affecting the endothelial isoform, and the thiophene-2-carboximidamide **4** is an interesting selective inhibitor of nNOS [22]. In addition, it has been previously published the discovery of dihydroisoquinolines **5** [23] and thienopyridines **6** [24], by replacement of the benzene ring by a thieno fused-ring that showed potent activity against iNOS, although the best compound produced hypotension *in vivo*. Moreover, Tinker et al. have demonstrated potency, selectivity, and efficacy in the rat adjuvant-induced arthritis model for 1,2-dihydro-4-quinazolinamine derivatives **7** and **8** [25] (Fig. 1).

Quinazoline and quinazolinone derivatives occur frequently in natural and synthetic pharmaceutical products which exhibit important biological properties. Quinazolinone is a building block for approximately 150 naturally occurring alkaloids such as glycosminine, echinozolinone, deoxyvascinone, rutaecarpine, etc. isolated from a number of plant families, animals, and from microorganisms [26].

Thus, quinazolinone scaffold has been incorporated into a number of therapeutic agents such as anticancer (raltitrexed), anticonvulsant (piriqualone), antihypertensive (ketanserin), antitussive (chloroqualone), diuretic (metolazone and quinethazone), and sedative-hypnotic (methaqualone), among others.

Nowadays, the quinazolinone ring system constitutes a key structural component in modern medicinal chemistry. Owing to its unique pharmaceutical significance, continuous advancements in the synthesis of new quinazolinone derivatives are an emerging source of research and development. Hence, efficient and reliable methods for their synthesis and fusion with many other bioactive molecules have led to the consideration of quinazolinone as a pivotal moiety in heterocyclic chemistry.

Previously, we have synthesized several heterocyclic families of NOS inhibitors such as 2-aminophenylpyrazolines **9** [27] and 2-aminophenylpyrroles **10** [28] (Fig. 1). In this paper, we describe the synthesis of a new type of derivatives where the free amine group belonging to the benzene ring is part of a new cycle of quinazolinone **11** that restricts the conformational freedom. Furthermore, their isosteric compounds with quinazolinthione **12** and quinazolinimine **13** structures have also been synthesized (Fig. 2). These heterocycles carry a guanidine moiety (existing in the NOS natural substrate L-arginine), or an urea or thiourea isosteric fragment forming part of a cycle. The synthetic route of these novel derivatives have been made by different cyclization methods from 2-aminobenzylamines, which were synthesized using both a traditional route and a flow hydrogenator. Also, we evaluate their activities as inhibitors of nNOS and iNOS isoforms in order to find new compounds with interesting pharmacological properties.

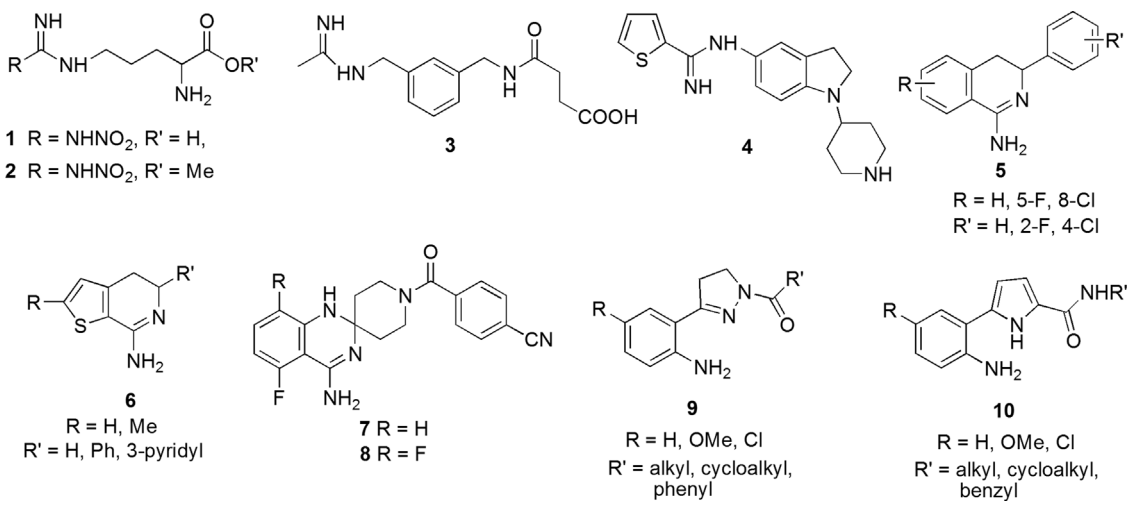
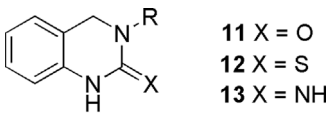


Figure 1. Examples of L-Arg analogs and other cyclic compounds as NOS inhibitors.



11 X = O
12 X = S
13 X = NH

R = linear alkyl, cycloalkyl, ramified alkyl, benzyl or acyl

Figure 2. General structure of derivatives **11**, **12**, and **13**.

Results and discussion

Chemistry

The general transformation of amines from aldehydes is an important method in organic synthesis because of their versatile utility as intermediates for preparation of pharmaceutical and agrochemical derivatives [29]. The reductive amination is commonly used for this transformation. The procedure involves the initial formation of a carbinolamine which dehydrates to give an imine. The second step needs a weakly acidic pH and the imine is protonated to form an iminium ion. Finally, subsequent reduction of this iminium cation produces the corresponding amino derivatives [30].

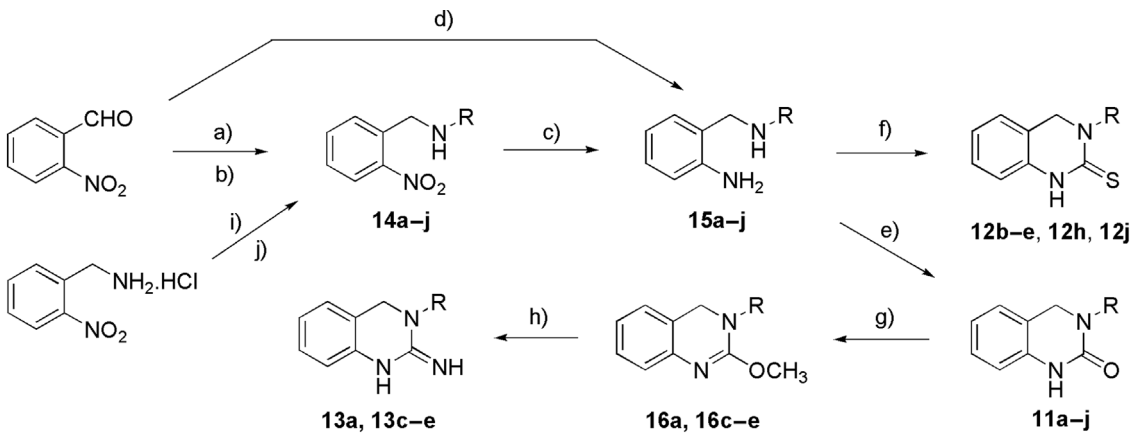
Scheme 1 shows the synthesis of derivatives **14a–i** using this methodology. The general procedure begins with the reductive amination of 2-nitrobenzaldehyde with alkyl-, cycloalkyl-, or benzyl-amines, via formation of the imino derivatives with sodium sulfate, and then reduction by sodium borohydride in methanol to yield the amino derivatives **14a–i** [31]. In a new stage, the reduction of the nitro group belonging to the aromatic ring, using $Fe/FeSO_4$ in water [32], leads to the diamine derivatives **15a–i**. In general, the yields of both reactions are good and the overall yields oscillate between 42 and 65% (Table 1).

While we performed the reductive amination of aldehydes with primary amines in the two steps previously described, we carried out the amine group reduction using a continuous flow-reactor (H-Cube flow hydrogenator model HC-2.SS) which employs a mixed hydrogen liquid flow stream. This methodology enabled to reduce the reaction times and to increase the yields (see Table 1). In the flow-reactor, the hydrogen is internally generated by the electrolysis of water and the gas-liquid mixture is pumped through a suitable catalyst contained in an interchangeable metal cartridge.

To carry out the studies of reductive amination, we have chosen the 2-nitrobenzaldehyde and the cyclopropylamine to obtain 2-((cyclopropylamino)methyl)benzenamine **15e**. An important prerequisite for flow chemistry is the choice of an appropriate solvent in order to avoid the precipitation of both reagents and products, which may lead to a blockage of the flow stream. In this case, out of the three solvents examined (MeOH, DCM, and THF), THF gave the best results under representative reaction conditions (10% Pd/C, full H_2, 40°C, 0.05 M, flow rate 0.5 mL/min).

The optimization of the flow conditions for the direct reductive amination and for the amino group reduction was systematically investigated by varying the flow rate, pressure, temperature, and the presence of acetic acid. The molar relation between the aldehyde and amine reagents was always 1:1.5 (Table 2).

The flow rate used was 1 or 0.5 mL/min. The increasing of the flow rate (run 1) causes the decrease of the yield, as a consequence of the residence time reduction in the flow-reactor. In this case, the yield of **15e** was low (10%) and we mainly isolated 2-aminobenzaldehyde (50%) and (2-aminophenyl)methanol (10%). These products indicated



Scheme 1. Synthesis of final compounds with general structures **11**, **12**, and **13**. (a) R-NH_2/Na_2SO_4, MeOH, 2 h rt; (b) $NaBH_4$/MeOH, 1 h rt; (c) $Fe/FeSO_4$, H_2O, 3 h reflux; (d) R-NH_2, 10% Pd/C, THF (flow hydrogenation); (e) $(Cl_3CO)_2CO$, Et_3N, DCM, 0°C, then 1 h rt, or CDI, THF, 3 h rt, then 18 h reflux; (f) Cl_2CS, Et_3N, Et_2O, −78°C, then rt, or TCDI, THF, h rt, then 18 h reflux; (g) $Me_3O^+BF_4^-$, DCM, 12 h rt; (h) $ClNH_4$, MeOH, 3.5 h reflux; (i) $Na_2CO_3.10H_2O$, H_2O, 10 min rt; (j) Ac_2O, Et_3N, DCM, 3 h rt.

Table 1. Yields of the nitrobenzylamines 14a–i, the diamines 15a–i obtained by conventional method, global yields of both reactions, and yields of the diamines 15a–h by the H-Cube flow hydrogenator.

Compound	Yield (%)	Compound	Yield (%)	Global yield (%)	Flow hydrogenator yield (%)	R
4a	63	**15a**	70	44	70	Me
14b	70	**15b**	60	42	70	Et
14c	85	**15c**	74	63	73	Pr
14d	73	**15d**	89	65	75	Bu
14e	51	**15e**	86	44	80	*c*-C_3H_5
14f	70	**15f**	80	56	80	*c*-C_4H_7
14g	73	**15g**	70	51	80	*c*-C_5H_9
14h	74	**15h**	70	52	78	*tert*-Bu
14i	80	**15i**	75	60	–	CH_2Ph

that the imine derivative was formed in little amount and the nitro and aldehyde groups were reduced to the amine and to the primary alcohol, respectively. When we reduced the flow rate to 0.5 mL/min (run 2), the yield of **15e** was increased from 10 to 60%. This fact showed that the imine derivative was obtained in better yield since the (2-aminophenyl)methanol derivative was not isolated. Then, we decided to change the full H_2 by 20 bar (with this H-Cube model, it is not possible to control the pressure in full H_2 mode) but we did not isolate the desired product (run 3). In another assay (run 4), we raised the temperature to 60°C but the yield decreased. This last result can be due to the partial evaporation of the cyclopropylamine.

On the other hand, an adequate pH is crucial for the formation of imine derivatives. The ideal pH is about 4.5. The second step needs acid pH but if the solution is very acid the amine is protonated, and as a consequence, the first step is inhibited. Thus, to increase the yield, we used a drop (20 μL) of glacial acetic acid with the aim of catalyzing the reaction (run 5). Before passing the reaction mixture through the H-Cube, the pH was about 5, and in this occasion, the yield increased up to 80%. Previously, Cho and Kang had published a convenient procedure for reductive amination using sodium borohydride activated by boric acid, *p*-toluenesulfonic acid monohydrate, or benzoic acid as reducing agents [33]. These acids, used to activate the reductive amination, are solid and we could not use them in a continuous flow reactor. This way, we decided to use acetic acid as an activator of the reductive amination.

We applied the optimized reaction conditions to the synthesis of the corresponding 2-aminobenzylamine derivatives **15a–h**. The compound **15i** (R = CH_2Ph) could not be synthesized by this methodology because of the loss of the benzyl group in the H-Cube flow hydrogenator. The results are shown in Table 1.

In all cases we obtained an optimum yield between 70 and 80%. In the experiments with methylamine and ethylamine, we have used a 2 M solution in THF of the corresponding amines. The smaller yields in these cases could be due to the lower boiling point and basicity of these amines compared to the rest of used amines.

3-Substituted-3,4-dihydroquinazolin-2(1*H*)-one derivatives **11a–i** were synthesized via ring closure of the appropriate diamines **15a–i**, either with triphosgene and triethylamine [34] or with 1,1′-carbonyldiimidazole (CDI) [35] as shown in the synthetic Scheme 1. The ring closure yield with triphosgene was usually low (40–44%); in contrast, refluxing an equimolar quantity of the diamine with CDI in THF gave the desired quinazolinones with a good yield (74–83%).

Furthermore, the arylthiourea derivatives **12b–e** were obtained either by treatment of the corresponding diamines **15b–e** with thiophosgene [36], or with 1,1′-(thiocarbonyl)-diimidazole (TCDI). The use of thiophosgene gave low yields (35%); however, when the cyclation took place with TCDI, the

Table 2. Flow hydrogenation optimization results for compound 15e at 0.05 M concentration.

Run	Acetic acid	Flow rate (mL/min)	Injection volume (mL)	Pressure	Temperature (°C)	Yield (%)
1	–	1	20	Full H_2	40	10
2	–	0.5	20	Full H_2	40	60
3	–	0.5	20	20 bar	40	–
4	–	0.5	20	Full H_2	60	50
5	1 drop	0.5	20	Full H_2	40	80

desired quinazolinthiones were obtained with better yields (75–84%), being the first time that this reactive is used in the synthesis of cyclic thioureas.

Finally, 3,4-dihydroquinazolin-2(1*H*)-imine derivatives **13a** and **13c–e** were synthesized via the corresponding intermediate dihydromethoxyquinazolines **16a** and **16c–e** by addition of trimethyloxonium tetrafluoroborate to the quinazolinones **11a** and **11c–e**, respectively, and then by treatment with NH_4Cl in methanol [37].

The derivatives **11j** and **12j** were synthesized by acylation of (2-nitrophenyl)methanamine hydrochloride with acetic anhydride [38], followed by reduction of the nitro group to give the intermediate **15j**, which was treated either with CDI in THF to give **11j**, or with TCDI to yield **12j** (Scheme 1).

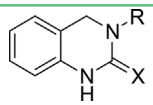
Biological results

The biological activities of the new heterocycles **11a–j**, **12b–e, 12h, 12j, 13a**, and **13c–e** as inhibitors of both iNOS and nNOS have been evaluated by means of *in vitro* assays using recombinant isoenzymes. We have carried out the assays using a 1 mM concentration of each compound in order to identify the more potent and selective derivatives. Table 3 shows the inhibition percentages versus nNOS and iNOS. The pyrrole **10a** (R = H, R′ = *c*-C_5H_9) previously described [28] and the known NOS inhibitor L-NAME [15] (Fig. 1) have been included as controls.

In general, these compounds behave as weak inhibitors against nNOS, since only three of them (**11a, 12d**, and **12h**) show a good percentage of inhibition at the concentration of 1 mM. Nevertheless, some conclusions can be inferred from the experimental data. From a qualitative point of view, the influence of R and X over the nNOS activity is variable. Most of the compounds with linear or ramified alkyl radicals in 3-position are better inhibitors than those with cycloalkyl, benzyl, or acyl ones, with some derivatives with methyl, butyl, and *tert*-butyl substituents being the best inhibitors (**11a**, 67.9%, **12d**, 60.4%, and **12h**, 70.9%, respectively).

Regarding the iNOS inhibition values, these compounds behave as better inhibitors against this isoform, since eight molecules show an inhibition percentage higher than 50%. In general, derivatives with a quinazolinone heterocycle are better inhibitors than those with quinazolinthione or quinazolinimine moieties, since four quinazolinones (**11a**, **11c**, **11e**, and **11f**) have good percentages of inhibition

Table 3. In vitro nNOS and iNOS inhibition (%) observed in the presence of 1 mM concentration of compounds 11a–j, 12b–e, 12h, 12j, 13a, and 13c–e. L-NAME and pyrrole 10a have been included as controls.



Compound	X	R	% nNOS inhibition[a)]	% iNOS inhibition[a)]
L-NAME[b)]			100.0 ± 1.03	77.01 ± 0.96
10[c)]			5.36 ± 3.19	52.79 ± 1.70
11a	O	Me	67.91 ± 0.45	58.86 ± 1.93
11b	O	Et	17.37 ± 0.16	41.27 ± 2.20
11c	O	Pr	2.21 ± 1.33	58.65 ± 3.45
11d	O	Bu	32.41 ± 2.45	34.27 ± 3.08
11e	O	*c*-C_3H_5	0.93 ± 3.22	72.74 ± 2.47
11f	O	*c*-C_4H_7	32.02 ± 2.38	57.44 ± 1.23
11g	O	*c*-C_5H_9	12.50 ± 0.77	26.22 ± 3.78
11h	O	*tert*-Bu	6.55 ± 1.62	36.45 ± 0.87
11i	O	CH_2Ph	11.49 ± 3.08	26.79 ± 1.79
11j	O	COMe	9.66 ± 1.23	22.01 ± 3.87
12b	S	Et	6.06 ± 3.81	20.38 ± 1.77
12c	S	Pr	11.91 ± 0.96	25.69 ± 3.48
12d	S	Bu	60.35 ± 1.80	59.83 ± 2.73
12e	S	*c*-C_3H_5	0.38 ± 1.5	10.16 ± 1.47
12h	S	*tert*-Bu	70.85 ± 3.55	55.35 ± 3.61
12j	S	COMe	14.42 ± 1.98	51.96 ± 2.48
13a	NH	Me	45.02 ± 1.76	57.53 ± 3.29
13c	NH	Pr	0.73 ± 2.38	27.19 ± 2.33
13d	NH	Bu	11.83 ± 1.95	32.99 ± 3.62
13e	NH	*c*-C_3H_5	11.53 ± 0.85	25.98 ± 0.67

[a)] Data represent the percentage of nNOS and iNOS inhibition produced by 1 mM concentration of each compound. Each value is the mean ± SEM of three experiments performed in triplicate using recombinant iNOS and nNOS enzymes. [b)] See [15]. [c)] See [31].

(between 57.4 and 72.7%), being **11e** (R = *c*-C_3H_5) the best inhibitor of this series and the most potent of all tested compounds. In addition, among derivatives with a sulfur atom, **12d** (R = Bu, 59.8%), **12h** (R = *tert*-Bu, 70.9%), and **12j** (R = COMe, 52.0%) are the best ones. Finally, among the quinazolinimines, **13a** (R = Me) is the best inhibitor of this series (57.5% of inhibition). Comparing these results with derivatives of general structure **10** previously described (the shown pyrrole **10a** is the best inhibitor of this family), the quinazolinones **11c** and **11e** are more potent and selective versus iNOS, demonstrating that the presence of an urea fragment in these heterocycles, which could mimic the guanidine moiety of the NOS natural substrate, is beneficial for the inhibition.

In general, these compounds show better inhibition values against iNOS than nNOS, what agrees with the published biological activities for the quinazoline derivatives **7**–**8** (Fig. 1), which are iNOS selective inhibitors. The most selective compounds are **11c** (iNOS/nNOS = 26.5) and **11e** (iNOS/nNOS = 78.2), which should be an interesting starting point for possible new alternatives in diseases where the iNOS is increased, such as septic shock, arthritis, and various kinds of inflammatory states.

Finally, compounds **11a**, **12d**, and **12h**, which are the best nNOS inhibitors, also show good iNOS percentages of inhibition, what could be useful in several illnesses where nitric oxide is increased due to both nNOS and iNOS isoforms, like Parkinson's disease.

Conclusions

In summary, in the current study, we have reported different strategies for the synthesis of a series of compounds which have a skeleton of quinazolinone, quinazolinthione, and quinazolinimine. We have demonstrated that the use of a flow hydrogenator for the synthesis of the intermediate 2-aminobenzylamines reduces to one the reaction steps, and the reductive amination activator acetic acid increases the overall yields. In addition, the ring closure has been achieved following two different methods in the quinazolinones as well as in the quinazolinthiones, being the first time that TCDI is used in the synthesis of this kind of cyclic compounds. Also, we report the results on nNOS and iNOS inhibition of final compounds. Among all derivatives, **12h** is the best nNOS inhibitor and **11e** is the iNOS most potent one of all tested compounds, and the most selective versus iNOS. This last quinazolinone could be a reference to design new inhibitors useful in inflammatory diseases such as septic shock or arthritis.

Experimental

Chemistry

Melting points were determined using an Electrothermal-1A-6301 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 300 spectrometer operating at 300.20 MHz for ^{1}H and 75.49 MHz for ^{13}C and on a Bruker ARX 400 spectrometer operating at 400.17 MHz for ^{1}H and 125.69 MHz for ^{13}C, in $CDCl_3$ (at concentration of ca. 27 mg/mL in all cases). The center of each peak of $CDCl_3$ [7.26 ppm (^{1}H) and 77.0 ppm (^{13}C)] was used as internal reference in a 5 mm ^{13}C/^{1}H dual probe (Wilmad, No. 528-PP). The temperature of the sample was maintained at 297 K. The peaks are reported in ppm (δ). High-resolution mass spectroscopy (HRMS) was carried out on a VG AutoSpec Q high-resolution mass spectrometer (Fision Instruments). Flash chromatography was carried out using silica gel 60, 230–240 mesh (Merck), and the solvent mixture reported within parentheses was used as eluent. The conventional organic solvents were purchased from Fisher and all other chemicals were purchased from Aldrich.

The InChI codes of the investigated compounds are provided as Supporting Information.

*General procedure for the synthesis of N-(2-nitrobenzyl)-alkylamine derivatives **14a–i***

To a cooled solution of 2-nitrobenzaldehyde (2 g, 13.23 mmol) in methanol (20 mL) was added Na_2SO_4 (2 g, 15.88 mmol) and the corresponding primary amine (1.2 mL, 14.55 mmol). After stirring at room temperature for 2 h, the precipitates were removed by filtration, and the solvent was evaporated. The residue (imine) was dissolved in dry methanol (20 mL) and treated with $NaBH_4$ (0.25 g, 6.61 mmol) at room temperature for 1 h. The mixture was concentrated, and the residue was dissolved in DCM (30 mL), washed with water, dried (Na_2SO_4), and concentrated to afford the crude mixture that was purified by flash chromatography (AcOEt/hexane 1:3).

*N-(2-Nitrobenzyl)methylamine (**14a**) [39, 40]*

Yellow oil, yield 1385 mg (63%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.95 (dd, 1H, $J_{3'\text{-}4'}$ = 8.1 Hz, $J_{3'\text{-}5'}$ = 1.2 Hz, H-3′), 7.60–7.53 (m, 2H, H-5′, H-6′), 7.43–7.39 (m, 1H, H-4′), 4.91 (bs, 1H, –NH–), 4.10 (s, 2H, Ph–C$\underline{H}_2$–), 2.46 (s, 3H, H-1). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 145.57 (C-2′), 136.12 (C-1′), 133.25 (C-5′), 131.43 (C-6′), 128.65 (C-4′), 125.14 (C-3′), 51.77 (Ph–$\underline{C}H_2$–), 35.36 (C-1).

*N-(2-Nitrobenzyl)ethylamine (**14b**) [40]*

Yellow oil, yield 1669 mg (70%). ^{1}H NMR (400.17 MHz, $CDCl_3$) δ: 7.90 (dd, 1H, $J_{3'\text{-}4'}$ = 8.1 Hz, $J_{3'\text{-}5'}$ = 1.3 Hz, H-3′), 7.57–7.54 (m, 2H, H-5′, H-6′), 7.38–7.34 (m, 1H, H-4′), 4.39 (bs, 1H, –NH–), 4.00 (s, 2H, Ph–C$\underline{H}_2$–), 2.66 (q, 2H, $J_{1\text{–}2}$ = 7.1 Hz, H-1), 1.08 (t, 3H, $J_{2\text{–}1}$ = 7.1 Hz, H-2). ^{13}C NMR (125.69 MHz, $CDCl_3$) δ: 149.05 (C-2′), 134.72 (C-1′), 133.27 (C-5′), 131.51 (C-6′), 128.17 (C-4′), 124.75 (C-3′), 50.13 (Ph–$\underline{C}H_2$–), 43.53 (C-1), 14.73 (C-2).

*N-(2-Nitrobenzyl)propylamine (**14c**) [40]*

Yellow oil, yield 2184 mg (85%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.99 (d, 1H, $J_{3'\text{-}4'}$ = 8.1 Hz, H-3′), 7.73–7.54 (m, 2H, H-5′, H-6′), 7.50–7.36 (m, 1H, H-4′), 4.08 (s, 2H, Ph–C$\underline{H}_2$–), 2.65 (t, 2H, $J_{1\text{–}2}$ = 7.2 Hz, H-1), 2.47 (bs, 1H, –NH–), 1.58 (m, 2H, H-2), 0.95 (t, 3H, $J_{3\text{–}2}$ = 7.4 Hz, H-3). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 146.07

(C-2′), 132.73 (C-1′), 131.33 (C-5′), 129.71 (C-6′), 128.35 (C-4′), 122.97 (C-3′), 51.30 (C-1), 49.65 (Ph–$\underline{C}H_2$–), 23.20 (C-2), 11.58 (C-3).

*N-(2-Nitrobenzyl)butylamine (**14d**) [41]*
Yellow oil, yield 2011 mg (73%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 8.00 (dd, 1H, $J_{3'\text{-}4'} = 8.1$ Hz, $J_{3'\text{-}5'} = 1.3$ Hz, H-3′), 7.71–7.59 (m, 2H, H-5′, H-6′), 7.48–7.42 (m, 1H, H-4′), 4.09 (s, 2H, Ph–$\underline{C}H_2$–), 2.69 (t, 2H, $J_{1\text{–}2} = 7.2$ Hz, H-1), 2.40 (bs, 1H, –NH–), 1.60–1.50 (m, 2H, H-2), 1.45–1.35 (m, 2H, H-3), 0.95 (t, 3H, $J_{3\text{–}2} = 7.3$ Hz, H-4). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 149.42 (C-2′), 135.50 (C-1′), 133.40 (C-5′), 131.69 (C-6′), 128.26 (C-4′), 124.97 (C-3′), 50.95 (Ph–$\underline{C}H_2$–), 32.30 (C-1), 29.93 (C-2), 20.58 (C-3), 14.09 (C-4).

*N-(2-Nitrobenzyl)cyclopropylamine (**14e**)*
Yellow oil, yield 1297 mg (51%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.95 (d, 1H, $J_{3'\text{-}4'} = 8.1$ Hz, H-3′), 7.59–7.57 (m, 2H, H-5′, H-6′), 7.45–7.39 (m, 1H, H-4′), 4.10 (s, 2H, Ph–$\underline{C}H_2$–), 2.49 (bs, 1H, –NH–), 2.16–2.11 (m, 1H, H-1), 0.47–0.40 (m, 4H, H-2, H-3). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 149.28 (C-2′), 135.56 (C-1′), 133.10 (C-5′), 131.88 (C-6′), 128.06 (C-4′), 124.79 (C-3′), 50.74 (Ph–$\underline{C}H_2$–), 30.07 (C-1), 6.56 (C-2, C-3).

*N-(2-Nitrobenzyl)cyclobutylamine (**14f**)*
Yellow solid, yield 1910 mg (70%). m.p.: 72–74°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.96 (d, 1H, $J_{3'\text{-}4'} = 8.3$ Hz, H-3′), 7.79–7.63 (m, 2H, H-5′, H-6′), 7.52–7.46 (m, 1H, H-4′), 3.99 (s, 2H, Ph–$\underline{C}H_2$–), 3.42–3.23 (m, 1H, H-1), 2.70 (bs, 1H, –NH–), 2.18–2.11, 1.75–1.69 (2m, 6H, H-2, H-3, H-4). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 142.01 (C-2′), 135.47 (C-1′), 133.55 (C-5′), 130.02 (C-6′), 128.37 (C-4′), 125.01 (C-3′), 54.15 (C-1), 48.42 (Ph–$\underline{C}H_2$–), 31.05 (C-2, C-4), 15.10 (C-3).

*N-(2-Nitrobenzyl)cyclopentylamine (**14g**)*
Yellow oil, yield 2127 mg (73%). ^{1}H NMR 300.20 MHz, $CDCl_3$) δ: 7.95 (d, 1H, $J_{3'\text{-}4'} = 8.1$ Hz, H-3′), 7.70–7.51 (m, 2H, H-5′, H-6′), 7.48–7.34 (m, 1H, H-4′), 4.03 (s, 2H, Ph–$\underline{C}H_2$–), 3.18–3.10 (m, 1H, H-1), 1.89–1.70 (m, 4H, H-2, H-5), 1.59–1.37 (m, 4H, H-3, H-4). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 147.72 (C-2′), 134.63 (C-1′), 131.66 (C-5′), 130.08 (C-6′), 126.27 (C-4′), 122.98 (C-3′), 58.04 (C-1), 48.04 (Ph–$\underline{C}H_2$–), 31.57 (C-2, C-5), 22.45 (C-3, C-4).

*N-(2-Nitrobenzyl)tert-butylamine (**14h**)*
Yellow oil, yield 2039 mg (74%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.93 (d, 1H, $J = 8.1$ Hz, H-3′), 7.71–7.56 (m, 2H, H-5′, H-6′), 7.43–7.38 (m, 1H, H-4′), 3.97 (s, 2H, Ph–$\underline{C}H_2$–), 1.20 (s, 9H, C($\underline{C}H_3$)$_3$). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 145.62 (C-2′), 135.29 (C-1′), 133.38 (C-5′), 129.76 (C-6′), 128.49 (C-4′), 124.67 (C-3′), 51.17 (C-1), 45.71 (Ph–$\underline{C}H_2$–), 28.65 (C($\underline{C}H_3$)$_3$).

*N-(2-Nitrobenzyl)benzylamine (**14i**) [40]*
Yellow oil, yield 2564 mg (80%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.99 (dd, 1H, $J_{3'\text{-}4'} = 8.1$ Hz, $J_{3'\text{-}5'} = 1.2$ Hz, H-3′), 7.71–7.58 (m, 2H, H-5′, H-6′), 7.48–7.29 (m, 6H, H-4′, H-2–H-6), 4.12 (s, 2H, Ph–$\underline{C}H_2$–), 3.88 (s, 2H, –$\underline{C}H_2$–Ph), 3.27 (bs, 1H, –NH–). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 138.96 (C-2′), 134.72 (C-1′), 133.46 (C-5′), 131.88 (C-6′), 129.20 (C-1), 128.71, 128.63 (C-2, C-3, C-5, C-6), 128.52 (C-4′), 127.66 (C-4), 125.07 (C-3′), 53.45 (Ph–$\underline{C}H_2$–), 50.04 (–$\underline{C}H_2$–Ph).

*General procedure for the preparation of N-(2-aminobenzyl)alkylamine derivatives **15a–i***
To a suspension of the corresponding nitroarene (0.524 mmol) in water, a mixture of Fe (5.24 mmol) and $FeSO_4$ (0.524 mmol) was added. The reaction was refluxed for 3 h, filtered through celite and extracted with DCM (3 × 15 mL) and ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (DCM/MeOH 9:1).

*N-(2-Aminobenzyl)methylamine (**15a**) [40]*
Yellow oil, yield 50 mg (70%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 6.99–6.80 (m, 2H, H-4′, H-6′), 6.64–6.55 (m, 2H, H-3′, H-5′), 3.98 (s, 2H, Ph–$\underline{C}H_2$–), 3.51 (bs, 3H, –NH_2, –NH–), 2.45 (s, 3H, H-1). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 146.50 (C-2′), 129.94, 128.45 (C-4′, C-6′), 123.13 (C-1′), 117.74, 115.68 (C-3′, C-5′), 49.50 (Ph–$\underline{C}H_2$–), 35.83 (C-1).

*N-(2-Aminobenzyl)ethylamine (**15b**) [40]*
Yellow oil, yield 47 mg (60%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.16–7.07 (m, 2H, H-4′, H-6′), 6.73–6.68 (m, 2H, H-3′, H-5′), 3.85 (s, 2H, Ph–$\underline{C}H_2$–), 2.73 (q, 2H, $J_{1\text{–}2} = 7.0$ Hz, H-1), 1.17 (t, 3H, $J_{2\text{–}1} = 7.0$ Hz, H-2). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 147.03 (C-2′), 130.38, 128.52 (C-4′, C-6′), 124.73 (C-1′), 117.94, 116.02 (C-3′, C-5′), 53.06 (Ph–$\underline{C}H_2$–), 44.03 (C-1), 15.61 (C-2).

*N-(2-Aminobenzyl)propylamine (**15c**) [40]*
Yellow oil, yield 64 mg (74%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.16–7.07 (m, 2H, H-4′, H-6′), 6.76–6.68 (m, 2H, H-3′, H-5′), 3.85 (s, 2H, Ph–$\underline{C}H_2$–), 3.73 (bs, 3H, –NH_2, –NH–), 2.65 (t, 2H, $J_{1\text{–}2} = 9.0$ Hz, H-1), 1.64–1.52 (m, 2H, H-2), 0.97 (t, 3H, $J_{3\text{–}2} = 9.0$ Hz, H-3). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 147.18 (C-2′), 130.17, 128.67 (C-4′, C-6′), 124.03 (C-1′), 117.98, 116.07 (C-3′, C-5′), 52.93 (C-1), 51.17 (Ph–$\underline{C}H_2$–), 23.13 (C-2), 12.04 (C-3).

*N-(2-Aminobenzyl)butylamine (**15d**) [41]*
Yellow oil, yield 84 mg (89%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.09–7.04 (m, 2H, H-4′, H-6′), 6.74–6.60 (m, 2H, H-3′, H-5′), 4.28 (s, 2H, Ph–$\underline{C}H_2$–), 2.70 (m, 1H, H-1), 1.70 (m, 2H, H-2), 1.28 (m, 2H, H-3), 0.70 (t, 3H, $J_{4\text{–}3} = 7.1$ Hz, H-4). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 147.18 (C-2′), 133.16, 130.64 (C-4′, C-6′), 117.78, 117.19 (C-3′, C-5′), 115.23 (C-1′), 47.57 (C-1), 45.09 (Ph–$\underline{C}H_2$–), 28.09 (C-2), 20.34 (C-3), 13.78 (C-4).

*N-(2-Aminobenzyl)cyclopropylamine (**15e**)*
Yellow oil, yield 73 mg (86%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.16–7.10 (m, 2H, H-4′, H-6′), 6.77–6.65 (m, 2H, H-3′, H-5′), 3.89 (s, 2H, Ph–$\underline{C}H_2$–), 3.62 (bs, 3H, –NH_2, –NH–), 2.21 (m, 1H, H-1), 0.52–0.43 (m, 4H, H-2, H-3). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 146.82 (C-2′), 130.59, 128.98 (C-4′, C-6′), 123.11 (C-1′),

118.26, 116.28 (C-3′, C-5′), 52.15 (Ph–$\underline{C}H_2$–), 30.59 (C-1), 6.01 (C-2, C-3).

*N-(2-Aminobenzyl)cyclobutylamine (**15f**)*
Yellow solid, yield 74 mg (80%). m.p.: 83–86°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.06–6.97 (m, 2H, H-4′, H-6′), 6.65–6.58 (m, 2H, H-3′, H-5′), 4.16 (bs, 3H, –NH_2, –NH–), 3.70 (s, 2H, Ph–$C\underline{H}_2$–), 3.29–3.24 (m, 1H, H-1), 2.17–2.08, 1.86–1.57 (2m, 6H, H-2, H-3, H-4). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 146.79 (C-2′), 131.00, 129.74 (C-4′, C-6′), 120.97 (C-1′), 118.11, 116.54 (C-3′, C-5′), 52.47 (C-1), 48.07 (Ph–$\underline{C}H_2$–), 30.51, 29.50 (C-2, C-4), 15.75 (C-3).

*N-(2-Aminobenzyl)cyclopentylamine (**15g**)*
Yellow oil, yield 70 mg (70%). ^{1}H NMR (400.17 MHz, $CDCl_3$) δ: 7.11–7.04 (m, 2H, H-4′, H-6′), 6.71–6.65 (m, 2H, H-3′, H-5′), 3.79 (s, 2H, Ph–$C\underline{H}_2$–), 3.14–3.11 (m, 1H, H-1), 1.88–1.38 (4m, 8H, H-2–H-5). ^{13}C NMR (125.69 MHz, $CDCl_3$) δ: 146.90 (C-2′), 129.69, 128.25 (C-4′, C-6′), 124.52 (C-1′), 117.72, 115.70 (C-3′, C-5′), 59.64 (C-1), 51.77 (Ph–$\underline{C}H_2$–), 33.20, 33.07 (C-2, C-5), 24.07, 23.99 (C-3, C-4).

*N-(2-Aminobenzyl)tert-butylamine (**15h**)*
Orange oil, yield 65 mg (70%). ^{1}H NMR (400.17 MHz, $CDCl_3$) δ: 7.12–7.07 (m, 2H, H-4′, H-6′), 6.74–6.67 (m, 2H, H-3′, H-5′), 3.81 (s, 2H, Ph–$C\underline{H}_2$–), 3.20 (bs, 3H, –NH_2, –NH–), 1.22 (s, 9H, $C(C\underline{H}_3)_3$). ^{13}C NMR (125.69 MHz, $CDCl_3$) δ: 146.89 (C-2′), 129.86, 128.39 (C-4′, C-6′), 124.27 (C-1′), 117.94, 116.01 (C-3′, C-5′), 51.26 (C-1), 45.62 (Ph–$\underline{C}H_2$–), 28.56 ($C(\underline{C}H_3)_3$).

*N-(2-Aminobenzyl)benzylamine (**15i**) [42]*
Yellow solid, yield 83 mg (75%). m.p.: 122–125°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.36–7.28 (m, 5H, H-2–H-6), 7.21–7.04 (m, 2H, C-4′, C-6′), 6.70–6.68 (m, 2H, C-3′, C-5′), 3.89, 3.84 (2s, 4H, 2 × Ph–$C\underline{H}_2$–). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 146.79 (C-2′), 138.25 (C-1), 131.00 (C-6′), 128.41, 128.20 (C-2, C-3, C-5, C-6), 128.37 (C-4′), 126.61 (C-4), 123.52 (C-1′), 117.11, 116.54 (C-3′, C-5′), 52.69 (–$\underline{C}H_2$Ph), 48.90 (Ph–$\underline{C}H_2$–).

*General procedure for the preparation of 2-((alkylamino)-methy)-lbenzenamine derivatives **15a–i** using a flow hydrogenator*
In a flask were dissolved 1 mmol of 2-nitrobenzaldehyde, 1.5 mmol of the corresponding primary amine, and 57 μL of the acetic acid glacial (synthesis grade) in 20 mL of dry THF. This mixture was passed through the flow hydrogenator (model HC-2.SS) using full H_2, 40°C, flow rate 0.5 mL/min and a cartridge of 10% Pd/C. Pre-packed cartridges (CatCart1) containing catalyst, are available from Thales Nanotechnology. In this study, 30 × 4 mm cartridges (CatCart130) were used throughout. Then, the solvent was evaporated and the residue was dissolved in DCM and washed with $NaHCO_3$ saturated solution and brine. The product was dried (Na_2SO_4) and concentrated to afford a residue that was purified by chromatography (AcOEt/Hexane 1:1 and then DCM/MeOH 9:1). The yields of these compounds are given in Table 1.

*Preparation of 3-alkyl- and 3-aryl-3,4-dihydroquinazolin-2(1H)-ones **11a–i***
Method A: A solution of triphosgene (0.624 mmol) in dichloromethane (3 mL) was added dropwise to a solution of the diamines **15a** and **15c** (1.248 mmol) and Et_3N (4.37 mmol) in DCM (10 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and was stirred for 1 h and then washed with H_2O (30 mL), $NaHCO_3$ saturated solution (30 mL), and brine (30 mL). The organic phase was separated and dried over Na_2SO_4. Filtration and evaporation of the solvent gave the crude mixture which was purified by flash chromatography (AcOEt/hexane 1:3) to yield the product.

Method B: To a stirred solution of the diamines **15b–i** (1.15 mmol) in 7.50 mL of THF was added CDI (1.43 mmol) and the solution was stirred at room temperature for 3 h. After 18 h of reflux, the solution was cooled and the solvent was evaporated. The crude was solved in DCM, washed with water, and dried over Na_2SO_4. Filtration and evaporation of the solvent gave the crude mixture which was purified by flash chromatography (ethyl acetate/hexane 1:3) to yield the product.

*3,4-Dihydro-3-methylquinazolin-2(1H)-one (**11a**) [43]*
White solid. Method A: Yield 44 mg (44%). m.p.: 148–152°C. ^{1}H NMR (400.17 MHz, $CDCl_3$) δ: 8.22 (bs, 1H, H-1), 7.17–7.13 (m, 1H, H-7), 7.01 (d, 1H, H-5, $J = 7.5$ Hz), 6.93–6.89 (m, 1H, H-6), 6.75 (d, 1H, H-8, $J = 7.9$ Hz), 4.44 (s, 2H, H-4), 3.04 (s, 3H, –CH_3). ^{13}C NMR (125.69 MHz, $CDCl_3$) δ: 154.82 (C-2), 137.19 (C-8a), 128.21 (C-7), 125.34 (C-5), 121.74 (C-6), 117.36 (C-4a), 113.85 (C-8), 50.92 (C-4), 34.56 (–CH_3). MS (LSIMS): *m/z* 185.7904 $[M+Na]^+$, calcd. mass for $C_9H_{10}N_2ONa$ 185.7901.

*3-Ethyl-3,4-dihydroquinazolin-2(1H)-one (**11b**)*
White solid. Method B: Yield 166 mg (82%). m.p.: 136–139°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 8.77 (bs, 1H, H-1), 7.17–7.14 (m, 1H, H-7), 7.03 (d, 1H, H-5, $J = 7.4$ Hz), 6.95–6.90 (m, 1H, H-6), 6.81 (d, 1H, H-8, $J = 7.9$ Hz), 4.46 (s, 2H, H-4), 3.54 (q, 2H, –$C\underline{H}_2$–CH_3, $J = 7.2$ Hz), 1.25 (t, 3H, –CH_2–$C\underline{H}_3$, $J = 7.2$ Hz). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 154.98 (C-2), 137.62 (C-8a), 128.11 (C-7), 125.54 (C-5), 121.68 (C-6), 117.51 (C-4a), 114.46 (C-8), 48.26 (C-4), 42.15 (–$\underline{C}H_2$–CH_3), 12.33 (–CH_2–$\underline{C}H_3$). MS (LSIMS): *m/z* 199.0799 $[M+Na]^+$, calcd. mass for $C_{10}H_{12}N_2ONa$ 199.0802.

*3,4-Dihydro-3-propylquinazolin-2(1H)-one (**11c**) [35]*
White solid. Method A: Yield 48 mg (40%). Method B: yield 164 mg (75%). m.p.: 111–113°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.84 (bs, 1H, H-1), 7.11–7.06 (m, 1H, H-7), 6.96 (d, 1H, H-5, $J = 7.6$ Hz), 6.88–6.83 (m, 1H, H-6), 6.66 (d, 1H, H-8, $J = 7.9$ Hz), 4.38 (s, 2H, H-4), 3.35 (t, 2H, –$C\underline{H}_2$–CH_2–CH_3, $J = 7.1$ Hz), 1.63–1.56 (m, 2H, –CH_2–$C\underline{H}_2$–CH_3), 0.89 (t, 3H, –CH_2–CH_2–$C\underline{H}_3$, $J = 7.4$ Hz). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 154.56 (C-2), 137.45 (C-8a), 128.17 (C-7), 125.40 (C-5), 121.89 (C-6), 117.63 (C-4a), 114.05 (C-8), 49.57, 48.57 (C-4, –$\underline{C}H_2$–CH_2–CH_3), 20.11 (–CH_2–$\underline{C}H_2$–CH_3), 11.05 (–CH_2–CH_2–$\underline{C}H_3$). MS (LSIMS): *m/z* 213.1008 $[M+Na]^+$, calcd. mass for $C_{11}H_{14}N_2ONa$ 213.1004.

*3-Butyl-3,4-dihydroquinazolin-2(1H)-one (**11d**)*
White solid. Method B: Yield 174 mg (74%). m.p.: 158–161°C. ^{1}H (300.20 MHz, $CDCl_3$) δ: 7.76 (bs, 1H, H-1), 7.21–7.16 (m, 1H, H-7), 7.06 (d, 1H, H-5, $J = 7.4$ Hz), 6.98–6.93 (m, 1H, H-6), 6.75 (d, 1H, H-8, $J = 7.9$ Hz), 4.48 (s, 2H, H-4), 3.48 (t, 2H, –C$\underline{H}_2$–CH_2–CH_2–CH_3, $J = 7.4$ Hz), 1.70–1.60 (m, 2H, –CH_2–C$\underline{H}_2$–CH_2–CH_3), 1.47–1.37 (m, 2H, –CH_2–CH_2–C$\underline{H}_2$–CH_3), 0.99 (t, 3H, –CH_2–CH_2–CH_2–C$\underline{H}_3$, $J = 7.3$ Hz). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 154.74 (C-2), 137.14 (C-8a), 128.45 (C-7), 125.64 (C-5), 122.15 (C-6), 117.78 (C-4a), 113.94 (C-8), 48.81, 47.21 (C-4, –$\underline{C}H_2$–CH_2–CH_2–CH_3), 29.28 (–CH_2–$\underline{C}H_2$–CH_2–CH_3), 20.27 (–CH_2–CH_2–C$\underline{H}_2$–CH_3), 14.12 (–CH_2–CH_2–CH_2–$\underline{C}H_3$). MS (LSIMS): *m/z* 227.1157 $[M+Na]^+$, calcd. mass for $C_{12}H_{16}N_2ONa$ 227.1160.

*3-Cyclopropyl-3,4-dihydroquinazolin-2(1H)-one (**11e**)*
Pallid brown solid. Method B: Yield 177 mg (79%). m.p.: 143–146°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 8.04 (bs, 1H, H-1), 7.21–7.16 (m, 1H, H-7), 7.07 (d, 1H, H-5, $J = 7.4$ Hz), 6.98–6.93 (m, 1H, H-6), 6.78 (d, 1H, H-8, $J = 7.9$ Hz), 4.44 (s, 2H, H-4), 2.71–2.65 (m, 1H, H-1$_{cycloprop.}$), 0.94–0.70 (m, 4H, H-2–H-3$_{cycloprop.}$). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 156.61 (C-2), 136.94 (C-8a), 128.35 (C-7), 125.53 (C-5), 120.81 (C-6), 118.75 (C-4a), 113.94 (C-8), 49.75 (C-4), 29.57 (C-1$_{cycloprop.}$), 7.68 (C-2–C-3$_{cycloprop.}$). MS (LSIMS): *m/z* 211.0806 $[M+Na]^+$, calcd. mass for $C_{11}H_{12}N_2ONa$ 211.0802.

*3-Cyclobutyl-3,4-dihydroquinazolin-2(1H)-one (**11f**)*
White solid. Method B: Yield 186 mg (80%). m.p.: 162–164°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.35 (sa, 1H, H-1), 7.11–7.08 (m, 1H, H-7), 7.03 (d, 1H, H-5, $J = 7.5$ Hz), 6.92–6.90 (m, 1H, H-6), 6.65 (d, 1H, H-8, $J = 7.9$ Hz), 4.79–4.76 (m, 1H, H-1$_{cyclobut.}$), 4.37 (s, 2H, H-4), 2.21–2.10 (m, 4H, H-2, H-4$_{cyclobut.}$), 1.68–1.61 (m, 2H, H-3$_{cyclobut.}$). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 154.85 (C-2), 137.21 (C-8a), 128.39 (C-7), 125.90 (C-5), 121.90 (C-6), 117.82 (C-4a), 113.34 (C-8), 49.33 (C-1$_{cyclobut.}$), 43.79 (C-4), 27.21 (C-2, C-4$_{cyclobut.}$), 14.82 (C-3$_{cyclobut.}$). MS (LSIMS): *m/z* 225.1008 $[M+Na]^+$, calcd. mass for $C_{12}H_{14}N_2ONa$ 225.1004.

*3-Cyclopentyl-3,4-dihydroquinazolin-2(1H)-one (**11g**) [44]*
Pallid yellow solid. Method B: Yield 192 mg (77%). m.p.: 157–160°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 8.75 (sa, 1H, H-1), 7.06–6.95 (m, 1H, H-7), 6.93 (d, 1H, H-5, $J = 7.5$ Hz), 6.92–6.90 (m, 1H, H-6), 6.83 (d, 1H, H-8, $J = 7.9$ Hz), 5.02–4.91 (m, 1H, H-1$_{cyclopent.}$), 4.35 (s, 2H, H-4), 1.90–0.77 (m, 8H, H-2–H-5$_{cyclopent.}$). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 156.89 (C-2), 138.76 (C-8a), 129.25 (C-7), 126.57 (C-5), 122.83 (C-6), 119.43 (C-4a), 115.29 (C-8), 56.23 (C-1$_{cyclopent.}$), 44.07 (C-4), 28.95 (C-2, C-5$_{cyclopent.}$), 25.88 (C-3, C-4$_{cyclopent.}$). MS (LSIMS): *m/z* 239.1155 $[M+Na]^+$, calcd. mass for $C_{13}H_{16}N_2ONa$ 239.1160.

*3-Tert-butyl-3,4-dihydroquinazolin-2(1H)-one (**11h**)*
White solid. Method B: Yield 162 mg (69%). m.p.: 148–150°C. ^{1}H NMR (400.17 MHz, $CDCl_3$) δ: 8.44 (bs, 1H, H-1), 7.17–7.13 (m, 1H, H-7), 7.04 (d, 1H, H-5, $J = 7.4$ Hz), 6.91–6.88 (m, 1H, H-6), 6.74 (d, 1H, H-8, $J = 7.8$ Hz), 4.39 (s, 2H, H-4), 1.51 (s, 9H, C(C$\underline{H}_3$)$_3$). ^{13}C NMR (125.69 MHz, $CDCl_3$) δ: 156.25 (C-2), 137.76 (C-8a), 128.07 (C-7), 125.16 (C-5), 121.24 (C-6), 119.39 (C-4a), 112.76 (C-8), 56.33 (C-1$_{tert\text{-}but}$), 44.70 (C-4), 28.48 (C($\underline{C}H_3$)$_3$). MS (LSIMS): *m/z* 205.1331 $[M+H]^+$, calcd. mass for $C_{12}H_{17}N_2O$ 205.1341.

*3-Benzyl-3,4-dihydroquinazolin-2(1H)-one (**11i**) [35]*
White solid. Method B: Yield 227 mg (83%). m.p.: 155–158°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.99 (sa, 1H, H-1), 7.97–7.29 (2m, 9H, H-5–H-8, H-2′–H-6′), 4.10 (s, 2H, H-4), 3.86 (s, 2H, –C$\underline{H}_2$Ph). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 154.76 (C-2), 136.99, 136.82 (C-8a, C-1′), 128.76, 128.26, 128.14, 127.64, 125.58, 121.92 (C-5–C-7, C-2′–C-6′), 117.51 (C-4a), 113.85 (C-8), 50.52 (C-4), 48.17 (–$\underline{C}H_2$Ph). MS (LSIMS): *m/z* 261.1005 $[M+Na]^+$, calcd. mass for $C_{15}H_{14}N_2ONa$ 261.1004.

*Preparation of 3-alkyl-3,4-dihydroquinazolin-2(1H)-thione derivatives **12b–e** and **12h***
Method A: A solution of the diamine **15b** (2.0 mmol) and 0.642 mL (4.61 mmol) of Et_3N was dissolved in 5 mL of dry Et_2O and was cooled to −78°C; to this was added, dropwise, a solution of 0.266 g (2.31 mmol) of thiophosgene solved in 5 mL of dry Et_2O, over a period of 15 min. The heterogeneous mixture was allowed to warm to room temperature and was filtered, and the solid was purified by chromatography (AcOEt/hexane 1:4) to yield the product.

Method B: To a stirred solution of the diamines **15b–e** and **15 h** (4.58 mmol) in 30 mL of THF, was added TCDI (5.73 mmol) and the solution was stirred at room temperature for 3 h. After 18 h of reflux, the solution was cooled and the solvent was evaporated. The crude was solved in DCM, washed with water, and dried over Na_2SO_4. Filtration and evaporation of the solvent gave the crude mixture which was purified by chromatography (AcOEt/hexane 1:4) to yield the product.

*3-Ethyl-3,4-dihydroquinazolin-2(1H)-thione (**12b**) [40]*
White solid. Method A: Yield 134 mg (35%). Method B: Yield 660 mg (75%). m.p.: 123–126°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 8.82 (bs, 1H, H-1), 7.23–7.17 (m, 1H, H-7), 7.05–6.98 (m, 2H, H-6, H-8), 6.82 (d, 1H, H-5, $J = 8.0$ Hz), 4.56 (s, 2H, H-4), 4.03 (q, 2H, –C$\underline{H}_2$–CH_3, $J = 7.2$ Hz), 1.34 (t, 3H, –CH_2–C$\underline{H}_3$, $J = 7.2$ Hz). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 175.94 (C-2), 134.81 (C-8a), 128.94 (C-7), 125.64 (C-8), 123.67 (C-6), 117.46 (C-4a), 113.62 (C-5), 49.07 (C-4), 29.93 (–$\underline{C}H_2$–CH_3), 11.42 (–CH_2–$\underline{C}H_3$). MS (LSIMS): *m/z* 193.0798 $[M+H]^+$, calcd. mass for $C_{10}H_{13}N_2S$ 193.0799.

*3,4-Dihydro-3-propylquinazolin-2(1H)-thione (**12c**) [40]*
White solid. Method B: Yield 765 mg (81%). m.p.: 122–124°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 8.42 (bs, 1H, H-1), 7.21–7.18 (m, 1H, H-7), 7.03–7.01 (m, 2H, H-6, H-8), 6.76 (d, 1H, H-5, $J = 7.9$ Hz), 4.54 (s, 2H, H-4), 3.91 (t, 2H, –C$\underline{H}_2$–CH_2–CH_3, $J = 7.6$ Hz), 1.83–1.78 (m, 2H, –CH_2–C$\underline{H}_2$–CH_3), 1.00 (t, 3H, –CH_2–CH_2–C$\underline{H}_3$, $J = 7.4$ Hz). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 175.44 (C-2), 134.53 (C-8a), 128.72 (C-7), 125.38 (C-8), 123.45 (C-6), 117.24 (C-4a), 113.24 (C-5), 55.43 (–$\underline{C}H_2$–CH_2–CH_3), 49.56

(C-4), 19.52 (–CH_2–$\underline{C}H_2$–CH_3), 11.15 (–CH_2–CH_2–$\underline{C}H_3$). MS (LSIMS): *m/z* 207.0950 $[M+H]^+$, calcd. mass for $C_{11}H_{15}N_2S$ 207.0956.

3-Butyl-3,4-dihydroquinazolin-2(1H)-thione ***(12d)***
Yellow solid. Method B: Yield 807 mg (80%). m.p.: 125–127°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 9.05 (bs, 1H, H-1), 7.11–7.05 (m, 1H, H-7), 6.93–6.86 (m, 2H, H-6, H-8), 6.77 (d, 1H, H-5, $J = 7.8$ Hz), 4.43 (s, 2H, H-4), 3.85 (t, 2H, –$\underline{C}H_2$–CH_2–CH_2–CH_3, $J = 7.7$ Hz), 1.70–1.60 (m, 2H, –CH_2–$\underline{C}H_2$–CH_2–CH_3), 1.38–1.26 (m, 2H, –CH_2–CH_2–$\underline{C}H_2$–CH_3), 0.89 (t, 3H, –CH_2–CH_2–CH_2–$\underline{C}H_3$, $J = 7.3$ Hz). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 176.24 (C-2), 135.10 (C-8a), 128.88 (C-7), 125.54 (C-8), 123.10 (C-6), 117.51 (C-4a), 113.68 (C-5), 53.77 (–$\underline{C}H_2$–CH_2–CH_2–CH_3), 49.60 (C-4), 28.36 (–CH_2–$\underline{C}H_2$–CH_2–CH_3), 20.26 (–CH_2–CH_2–$\underline{C}H_2$–CH_3), 14.28 (–CH_2–CH_2–CH_2–$\underline{C}H_3$). MS (LSIMS): *m/z* 221.1098 $[M+H]^+$, calcd. mass for $C_{12}H_{17}N_2S$ 221.1102.

3-Cyclopropyl-3,4-dihydroquinazolin-2(1H)-thione ***(12e)***
Yellow solid. Method B: Yield 786 mg (84%). m.p.: 156–158°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 9.04 (bs, 1H, H-1), 7.19–7.16 (m, 1H, H-7), 7.03–6.97 (m, 2H, H-6, H-8), 6.83 (d, 1H, H-5, $J = 7.8$ Hz), 4.47 (s, 2H, H-4), 3.04–3.00 (m, 1H, H-$1_{cycloprop.}$), 1.04–0.83 (2m, 4H, H-2, H-$3_{cycloprop.}$). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 179.85 (C-2), 134.37 (C-8a), 128.63 (C-7), 125.26 (C-8), 123.47 (C-6), 118.36 (C-4a), 113.31 (C-5), 50.46 (C-4), 34.81 (C-$1_{cycloprop.}$), 9.61 (C-2, C-$3_{cycloprop.}$). MS (LSIMS): *m/z* 205.0796 $[M+H]^+$, calcd. mass for $C_{11}H_{13}N_2S$ 205.0799.

3-Tert-butyl-3,4-dihydroquinazolin-2(1H)-thione ***(12h)***
Yellow solid. Method B: Yield 962 mg (72%). m.p.: 163–165°C. ^{1}H NMR (400.17 MHz, $CDCl_3$) δ: 8.70 (bs, 1H, H-1), 7.24–7.22 (m, 1H, H-7), 7.12–7.04 (m, 2H, H-6, H-8), 6.82 (d, 1H, H-8, $J = 7.8$ Hz), 4.48 (s, 2H, H-4), 1.82 (s, 9H, C($C\underline{H}_3$)$_3$). ^{13}C NMR (125.69 MHz, $CDCl_3$) δ: 179.23 (C-2), 135.70 (C-8a), 128.61 (C-7), 124.77 (C-8), 123.18 (C-6), 120.10 (C-4a), 112.39 (C-5), 60.96 (C-$1_{tert\text{-}but}$), 46.13 (C-4), 29.10 (C($\underline{C}H_3$)$_3$). MS (LSIMS): *m/z* 221.1109 $[M+H]^+$, calcd. mass for $C_{12}H_{17}N_2S$ 221.1112.

Preparation of 3-alkyl-3,4-dihydro-2-methoxyquinazoline derivatives ***16a*** *and* ***16c–e***
To a magnetically stirred slurry of trimethyloxonium tetrafluoroborate (4.77 mmol) in DCM (20 mL) under argon was added the 3,4-dihydroquinazolin-2(1*H*)-ones **9a** and **9c–e** (3.98 mmol). This mixture was stirred at room temperature for 12 h and then it was diluted with 15 mL of DCM, washed with 40 mL of $NaHCO_3$ saturated solution, and dried (Na_2SO_4). Filtration and evaporation of the solvent gave the crude mixture which was purified by chromatography (AcOEt/hexane 1:6) to yield the product.

3,4-Dihydro-2-methoxy-3-methylquinazoline ***(16a)***
White oil, yield 583 mg (83%). ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.19–6.92 (3m, 4H, H-5–H-8), 4.48 (s, 2H, H-4), 3.92 (s, 3H, –OCH_3), 2.92 (s, 3H, –CH_3). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 156.97 (C-2), 144.29 (C-8a), 128.41, 124.91, 123.01, 122.69 (C-5–C-8), 121.24 (C-4a), 54.29 (–OCH_3), 52.61 (C-4), 35.49 (–CH_3).

3,4-Dihydro-2-methoxy-3-propylquinazoline ***(16c)***
White solid, yield 569 mg (70%). m.p.: 116–119°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.09–6.83 (3m, 4H, H-5–H-8), 4.45 (s, 2H, H-4), 3.86 (s, 3H, –OCH_3), 3.17 (t, 2H, –$\underline{C}H_2$–CH_2–CH_3, $J = 7.3$ Hz), 1.57–1.51 (m, 2H, –CH_2–$\underline{C}H_2$–CH_3), 0.85 (t, 3H, –CH_2–CH_2–$\underline{C}H_3$, $J = 7.5$ Hz). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 150.86, 149.92 (C-2, C-8a), 128.38, 124.94, 122.80, 122.67 (C-5–C-8), 122.30 (C-4a), 54.34 (–OCH_3), 50.30, 50.09 (C-4, –$\underline{C}H_2$–CH_2–CH_3), 20.46 (–CH_2–$\underline{C}H_2$–CH_3), 11.44 (–CH_2–CH_2–$\underline{C}H_3$).

3-Butyl-3,4-dihydro-2-methoxyquinazoline ***(16d)***
White solid, yield 634 mg (73%). m.p.: 120–123°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.18–6.92 (3m, 4H, H-5–H-8), 4.55 (s, 2H, H-4), 3.93 (s, 3H, –OCH_3), 3.30 (t, 2H, –$\underline{C}H_2$–CH_2–CH_2–CH_3, $J = 7.3$ Hz), 1.62–1.57 (m, 2H, –CH_2–$\underline{C}H_2$–CH_2–CH_3), 1.41–1.31 (m, 2H, –CH_2–CH_2–$\underline{C}H_2$–CH_3), 0.97 (t, 3H, –CH_2–CH_2–CH_2–$\underline{C}H_3$, $J = 7.3$ Hz). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 156.67 (C-2), 144.08 (C-8a), 128.36, 124.92, 122.79, 122.66 (C-5–C-8), 121.10 (C-4a), 54.33 (–OCH_3), 50.27, 48.14 (C-4, –$\underline{C}H_2$–CH_2–CH_2–CH_3), 29.60 (–CH_2–$\underline{C}H_2$–CH_2–CH_3), 20.20 (–CH_2–CH_2–$\underline{C}H_2$–CH_3), 14.05 (–CH_2–CH_2–CH_2–$\underline{C}H_3$).

3-Cyclopropyl-3,4-dihydro-2-methoxyquinazoline ***(16e)***
Pallid brown solid, yield 724 mg (90%). m.p.: 114–117°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.24–6.95 (3m, 4H, H-5–H-8), 4.45 (s, 2H, H-4), 3.86 (s, 3H, –OCH_3), 2.51–2.47 (m, 1H, H-$1_{cycloprop.}$), 0.89–0.61 (2m, 4H, H-2, H-$3_{cycloprop.}$). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 158.51 (C-2), 143.78 (C-8a), 127.51, 124.59, 123.50, 122.93 (C-5–C-8), 122.85 (C-4a), 54.58 (–OCH_3), 50.66 (C-4), 29.56 (H-$1_{cycloprop.}$), 8.07 (H-2, H-$3_{cycloprop.}$).

Preparation of 3-alkyl-3,4-dihydroquinazolin-2(1H)-imine derivatives ***13a*** *and* ***13c–e***
A mixture of the dihydroquinazolines **16a** and **16c–e** (1.43 mmol) and NH_4Cl (0.16 g, 1.57 mmol) was solved in methanol (20 mL) and was stirred under reflux for 3.5 h. After this time, the solvent was evaporated. The crude mixture was purified by chromatography (AcOEt/hexane 1:1) to yield the product.

3,4-Dihydro-3-methylquinazolin-2(1H)-imine ***(13a)***
White solid, yield 150 mg (65%). m.p.: 193–195°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.84 (bs, 2H, H-1, =NH), 7.22–7.16 (m, 1H, H-7), 7.06 (d, 1H, H-5, $J = 7.5$ Hz), 6.98–6.93 (m, 1H, H-6), 6.76 (d, 1H, H-8, $J = 7.9$ Hz), 4.49 (s, 2H, H-4), 3.07 (s, 3H, –CH_3). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 137.25 (C-2), 136.35 (C-8a), 128.78 (C-7), 125.62 (C-5), 122.09 (C-6), 117.59 (C-4a), 114.00 (C-8), 51.13 (C-4), 34.80 (–CH_3). MS (LSIMS): *m/z* 162.1097 $[M+H]^+$, calcd. mass for $C_9H_{12}N_3$ 162.1001.

3,4-Dihydro-3-propylquinazolin-2(1H)-imine ***(13c)***
White solid, yield 168 mg (62%). m.p.: 194–197°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.09–7.06 (m, 1H, H-7), 6.96 (d, 1H, H-5,

$J = 7.5$ Hz), 6.88–6.83 (m, 1H, H-6), 6.59 (d, 1H, H-8, $J = 7.9$ Hz), 4.37 (s, 2H, H-4), 3.33 (t, 2H, –C$\underline{H}_2$–CH_2–CH_3, $J = 7.0$ Hz), 1.66–1.55 (m, 2H, –CH_2–C$\underline{H}_2$–CH_3), 0.88 (t, 3H, –CH_2–CH_2–C$\underline{H}_3$, $J = 7.0$ Hz). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 137.25 (C-2), 136.20 (C-8a), 128.44, 125.71, 122.09 (C-5–C-7), 117.93 (C-4a), 113.66 (C-8), 48.83 (C-4), 29.95 (–$\underline{C}H_2$–CH_2–CH_3), 20.44 (–CH_2–$\underline{C}H_2$–CH_3), 11.46 (–CH_2–CH_2–$\underline{C}H_3$). MS (LSIMS): *m/z* 190.1297 $[M+H]^+$, calcd. mass for $C_{11}H_{16}N_3$ 190.1302.

*3-Butyl-3,4-dihydroquinazolin-2(1H)-imine (**13d**)*
White solid, yield 198 mg (68%). m.p.: 194–197°C. ^{1}H NMR (400.17 MHz, $CDCl_3$) δ: 8.52 (s, 2H, H-1, =NH), 7.15–7.12 (m, 1H, H-7), 7.04 (d, 1H, H-5, $J = 7.4$ Hz), 6.91–6.88 (m, 1H, H-6), 6.76 (d, 1H, H-8, $J = 7.9$ Hz), 4.43 (s, 2H, H-4), 3.45 (t, 2H, –C$\underline{H}_2$–CH_2–CH_2–CH_3, $J = 7.4$ Hz), 1.65–1.59 (m, 2H, –CH_2–C$\underline{H}_2$–CH_2–CH_3), 1.42–1.35 (m, 2H, –CH_2–CH_2–C$\underline{H}_2$–CH_3), 0.96 (t, 3H, –CH_2–CH_2–CH_2–C$\underline{H}_3$, $J = 7.4$ Hz). ^{13}C NMR (125.69 MHz, $CDCl_3$) δ: 154.83 (C-2), 137.36 (C-8a), 128.05 (C-7), 125.24 (C-5), 121.51 (C-6), 117.52 (C-4a), 113.75 (C-8), 48.55 (C-4), 46.77 (–C$\underline{H}_2$–CH_2–CH_2–CH_3), 29.06 (–CH_2–$\underline{C}H_2$–CH_2–CH_3), 20.02 (–CH_2–CH_2–$\underline{C}H_2$–CH_3), 13.88 (–CH_2–CH_2–CH_2–$\underline{C}H_3$). MS (LSIMS): *m/z* 204.1502 $[M+H]^+$, calcd. mass for $C_{12}H_{18}N_3$ 204.1500.

*3-Cyclopropyl-3,4-dihydroquinazolin-2(1H)-imine (**13e**)*
Pallid brown solid, yield 174 mg (65%). m.p.: 194–197°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 7.18–7.13 (m, 1H, H-7), 7.05 (d, 1H, H-5, $J = 7.4$ Hz), 6.95–6.91 (m, 1H, H-6), 6.68 (d, 1H, H-8, $J = 7.6$ Hz), 4.41 (s, 2H, H-4); 2.67–2.60 (m, 1H, H-$1_{cycloprop.}$), 0.90–0.66 (2m, 4H, H-2, H-$3_{cycloprop.}$). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 156.26 (C-2), 136.23 (C-8a), 128.16 (C-7), 125.32 (C-5), 121.88 (C-6), 118.53 (C-4a), 113.56 (C-8), 49.49 (C-4), 29.30 (C-$1_{cycloprop.}$), 7.48 (C-2, C-$3_{cycloprop.}$). MS (LSIMS): *m/z* 188.1185 $[M+H]^+$, calcd. mass for $C_{11}H_{14}N_3$ 188.1188.

*Preparation of 3-acetyl-3,4-dihydroquinazolin-2(1H)-one **11j**, and 3-acetyl-3,4-dihydroquinazolin-2(1H)-thione **12j***

*N-(2-Nitrobenzyl)acetamide (**14j**) [38]*
To a solution of (2-nitrophenyl)methanamine hydrochloride (1.68 g, 8.91 mmol) in water (20 mL) was added $Na_2CO_3.10\,H_2O$ (5.94 g, 20.76 mmol), and was stirred while 10 min. The solution was washed with DCM, brine, and dried. The crude (2-nitrophenyl)methanamine was solved in 20 mL DCM, and added Et_3N (2.05 mL, 11.59 mmol), and acetic anhydride (1.40 mL, 11.59 mmol). The reaction mixture was stirred to room temperature for 3 h, washed with 2 N HCl, 2 M NaOH, H_2O, and brine. The organic phase was dried (Na_2SO_4) and filtered. Evaporation of the solvent rendered a residue that was purified by flash chromatography (AcOEt/hexane 4:1) to yield the product. White solid, yield 1214 mg (84%). m.p.: 99–101°C. ^{1}H NMR (400.17 MHz, $CDCl_3$) δ: 8.05 (d, 1H, H-3′, $J = 8.3$ Hz); 7.67–7.59 (m, 2H, H-4′, H-5′); 7.47–7.43 (m, 1H, H-6′); 6.43 (bs, 1H, –NH–); 4.65 (d, 2H, –CH_2–, $J = 3.9$ Hz); 1.99 (s, 3H, H-2). ^{13}C NMR (125.69 MHz, $CDCl_3$) δ: 172.79 (C-1); 136.72 (C-2′), 136.31 (C-1′); 135.22 (C-5′, C-6′); 131.38, 127.67 (C-3′, C-4′); 43.88 (–CH_2–); 25.88 (C-2).

*N-(2-Aminobenzyl)acetamide (**15j**) [45]*
Following the general method described for the compounds **15a–i** it was obtained a residue which was purified by chromatography (AcOEt/hexane 1:1) to yield the product. White solid, yield 52 mg (60%). m.p.: 114–116°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) 7.16–7.04 (m, 2H, H-4′, H-6′), 6.73–6.68 (m, 2H, H-3′, H-5′), 5.92 (bs, 1H, –NH–), 4.38 (d, 2H, –CH_2–, $J = 6.2$ Hz), 4.23 (bs, 2H, –NH_2), 2.01 (s, 3H, H-2). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 170.77 (C-1), 145.84 (C-2′), 130.82, 129.54 (C-4′, C-6′), 122.13 (C-1′), 117.98, 115.97 (C-3′, C-5′), 41.14 (–CH_2–); 23.42 (C-2).

*3-Acetyl-3,4-dihydroquinazolin-2(1H)-one (**11j**)*
Following method B for the synthesis of compounds **11a–i**, and using the 2-aminobenzylacetamide **15j** (0.204 g, 1.242 mmol), it was obtained a residue which was purified by chromatography (AcOEt/hexane 1:4) to yield the product. White solid, yield 178 mg (75%). m.p.: 150–153°C. ^{1}H NMR (300.20 MHz, $CDCl_3$) δ: 8.41 (bs, 1H, H-1), 7.30–7.21 (m, 2H, H-5, H-7), 7.10–7.05 (m, 1H, H-6), 6.87 (d, 1H, H-8, $J = 7.8$ Hz), 4.94 (s, 2H, H-4), 2.65 (s, 3H, –CH_3). ^{13}C NMR (75.49 MHz, $CDCl_3$) δ: 172.67 (–$\underline{C}OCH_3$), 153.72 (C-2), 135.77 (C-8a), 128.85, 126.27, 123.66 (C-5–C-7), 119.82 (C-4a), 114.20 (C-8), 44.16 (C-4), 26.84 (–CH_3). MS (LSIMS): *m/z* 213.0600 $[M+Na]^+$, calcd. mass for $C_{10}H_{10}N_2O_2Na$ 213.0603.

*3-Acetyl-3,4-dihydroquinazolin-2(1H)-thione (**12j**)*
Following method B for the synthesis of compounds **12b–e** and **12h**, and using the 2-aminobenzylacetamide **15j** (0.328 g, 2 mmol), it was obtained a crude product which was purified by chromatography (AcOEt/hexane 1:4) to yield the product. White solid, yield 289 mg (70%). m.p.: 123–125°C. ^{1}H NMR (400.17 MHz, $CDCl_3$) δ: 7.42–7.26 (2m, 4H, H-5–H-8), 6.06 (bs, 3H, H-1), 4.50 (s, 2H, H-4), 2.07 (s, 3H, –CH_3). ^{13}C NMR (125.69 MHz, $CDCl_3$) δ: 175.23 (C-2), 172.81 (–$\underline{C}OCH_3$), 136.69 (C-8a), 132.34, 131.39, 130.25, 129.49 (C-5–C-8), 125.51 (C-4a), 43.00 (C-4), 25.90 (–CH_3). MS (LSIMS): *m/z* 229.0402 $[M+Na]^+$, calcd. mass for $C_{10}H_{10}N_2OSNa$ 229.0411.

In vitro nNOS and iNOS activities determination

L-Arginine, L-citrulline, *N*-(2-hydroxymethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), DL-dithiothreitol (DTT), hypoxantine-9-β-D-ribofuranosid (inosine), ethylene glycol-bis-(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), bovine serum albumin (BSA), Dowex-50 W (50 × 8–200), FAD, NADPH, and 5,6,7,8-tetrahydro-L-biopterin dihydrocloride (H_4-biopterin) were obtained from Sigma–Aldrich Química (Spain). L-[^{3}H]-Arginine (54 Ci/mmol) was obtained from Amersham (Amersham Biosciences, Spain). Tris-(hydroxymethyl)-aminomethane (Tris-HCl) and calcium chloride were obtained from Merck (Spain). Calmodulin from bovine brain, and recombinant iNOS and nNOS were obtained from Alexis Biochemicals (Enzo Life Sciences, Grupo Taper, Seville, Spain).

The nNOS activity was measured by the Bredt and Snyder [46] method, monitoring the conversion of L-[^{3}H]-arginine to L-[^{3}H]-citrulline. The final incubation volume was

100 μL and consisted of 10 μL of an aliquot of recombinant nNOS added to a buffer with a final concentration of 25 mM Tris-HCl, 1 mM DTT, 4 μM H_4-biopterin, 10 μM FAD, 0.5 mM inosine, 0.5 mg/mL BSA, 0.1 mM $CaCl_2$, 10 μM L-arginine, and 50 nM L-[^{3}H]-arginine, at pH 7.6. The reaction was started by the addition of 10 μL of NADPH (0.75 mM final) and 10 μL of each 3,4-dihydroquinazolin-2(1*H*)-one, -thione or -imine derivative in ethanol 10% to give a final concentration of 1 mM. The tubes were vortexed and incubated at 37°C for 30 min. Control incubations were performed by the omission of NADPH. The reaction was halted by the addition of 400 μL of cold 0.1 M HEPES, 10 mM EGTA, and 0.175 mg/mL L-citrulline, pH 5.5. The reaction mixture was decanted into a 2 mL column packed with Dowex-50 W ion-exchange resin (Na^+ form) and eluted with 1.2 mL of water. L-[^{3}H]-Citrulline was quantified by liquid scintillation spectroscopy. The retention of L-[^{3}H]-arginine in this process was greater than 98%. Specific enzyme activity was determined by subtracting the control value, which usually amounted to less than 1% of the radioactivity added. The nNOS activity was expressed as picomoles of L-[^{3}H]-citrulline produced (/mg of protein/min).

For iNOS activity determination, the procedure was essentially the same as for nNOS, excepting that an aliquot of 10 μL recombinant iNOS was used instead of nNOS, and the mixture was incubated in the absence of calmodulin.

Statistical analysis

Data are expressed as the mean ± SEM. One-way analysis of variance, followed by the Newmane-Keuls multiple range test was used. A $P < 0.05$ was considered statistically significant.

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