Bioorganic & Medicinal Chemistry 17 (2009) 3713–3719

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis, antiplatelet and in silico evaluations of novel N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides

Alessandro K. Jordão [a], Vitor F. Ferreira [a,*], Emerson S. Lima [b], Maria C. B. V. de Souza [a], Eduardo C. L. Carlos [b], Helena C. Castro [c], Reinaldo B. Geraldo [c], Carlos R. Rodrigues [c], Maria C. B. Almeida [a], Anna C. Cunha [a,*]

[a] *Universidade Federal Fluminense, Departamento de Química Orgânica, Outeiro de São João Batista, s/n°, Niterói, 24020-141 Rio de Janeiro, Brazil*
[b] *Universidade Federal do Amazonas, Faculdade de Ciências Farmacêuticas, Rua Alexandre Amorin, 330, Manaus, 69010-300 Amazonas, Brazil*
[c] *Universidade Federal Fluminense, Departamento de Biologia Celular e Molecular, LABioMol, Niterói, 24020-141 Rio de Janeiro, Brazil*

ARTICLE INFO

Article history:
Received 12 January 2009
Revised 23 March 2009
Accepted 25 March 2009
Available online 1 April 2009

Keywords:
Triazoles
Diazo compounds
Antiplatelet properties
In silico evaluation

ABSTRACT

This paper describes the synthesis, antiplatelet and theoretical evaluations of 10 N-substituted-phenylamino-5-methyl-*1H*-1,2,3-triazole-4-carbohydrazides (**2a–j**). These compounds were synthesized, characterized and screened for their in vitro antiplatelet profile against human platelet aggregation using arachidonic acid, adrenaline and ADP as agonists. Among *NAH* derivatives **2a–j**, the compounds **2a**, **2c**, **2e**, **2g** and **2h** were the most promising molecules with significant antiplatelet activity.

1. Introduction

The platelets play a major role in the pathogenesis of thrombotic disorders that are activated by a number of metabolic pathways.[1–3] These pathways lead to the production of pro-aggregatory molecules such as thromboxane A_2 (TXA_2) and adenosine diphosphate (ADP) that are released from platelet granules. These molecules amplify platelet responses to collagen and recruit additional platelets to the site of injury.[2–5] In addition to these agonists, there are adrenaline and noradrenaline that act on the alpha-adrenergic receptor in human platelets with a weaker pro-aggregatory profile but increasing the platelet sensitivity to other agonists such as thrombin. Adrenaline and noradrenaline are involved in stress situations that in this new millennium might contribute on causing heart attacks, stroke and vascular incidents.[6]

Among the physiological agonists that activate platelets, arachidonic acid metabolites are of particular relevance (Fig. 1).[7,8] Arachidonic acid is metabolized to TXA_2 and 12-hydroxyeicosatetraenoic (12-HETE) through two catalytic pathways mediated by cyclooxygenase (a prostaglandin endoperoxide H synthase—PGHS), and lipoxygenase, respectively.[7,8] The increasing of TXA_2 concentration has been linked to cardiovascular diseases including acute myocardial ischemia and heart failure. Currently, the inhibition of TXA_2 production by acetylsalicylic acid (ASA), also known as aspirin, is widely used to reduce the risk of myocardial infarction and thromboembolic disease. The mechanism of action of ASA is related to its capacity to permanently inactivate the platelet PGHS by acetylation of the serine aminoacid residue.[9,10] However, collateral effects and resistance to aspirin are fully described in literature.[7,8,11]

The antiplatelet therapy has been widely recognized as of potential medical application to reduce the incidence of stroke, heart attacks and death from vascular causes in individuals with symptomatic atherothrombotic diseases.[12] Stroke is responsible for at least 5–7 million deaths and 50–58 millions stroke related-disability. According to World Health Organization, 87% of these deaths were in low-income and middle-income countries and without intervention, the number of global deaths should rise to 6.5 million in 2015 and to 7.8 million in 2030.[13]

Recently, we described the synthesis of N-substituted-phenyl-1,2,3-triazole-4-acylhydrazones with antiplatelet activity.[14] These compounds inhibited the arachidonic acid-induced platelet aggregation in rabbit and human citrated platelet rich plasma.[14] From this study we identified the antiplatelet compound *N′*-[(4′-bromo-phenyl)methylene)]-1-(*p*-chlorophenyl)-1*H*-1,2,3-triazole-4-carbohydrazide (**1**) (Fig. 1), which presented a similar profile to aspirin without the undesired gastric ulceration symptoms. Studies also showed that hydrazone[14,16–19] group may play an important role for the antithrombotic activity.

In the search for new triazole derivatives with antiplatelet activity and to perform a structure–activity relationship study, we describe herein the synthesis and antiplatelet evaluation of new substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides (**2**), in which the 4-bromophenyl moiety (subunit

* Corresponding authors. Tel.: +55 21 26292364; fax: +55 21 26292135 (A.C.C.).
E-mail address: annac@vm.uff.br (A.C. Cunha).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.03.053

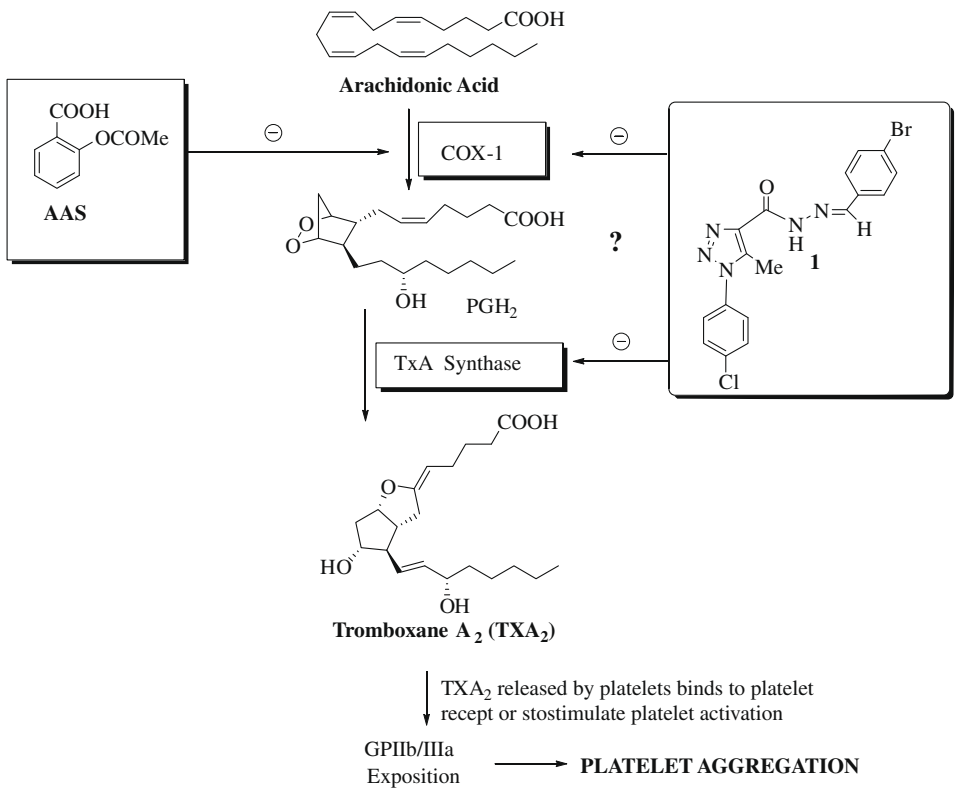


Figure 1. Arachidonic acid (AA) metabolism involved in the platelets aggregation process. Arachidonic acid is metabolized through cyclooxygenase (COX) and thromboxane A_2 (TXA_2) synthase generating PGH_2 and thromboxane A_2 (TXA_2), respectively. Aspirin (AAS) is able to inhibit COX. The feasible targets in the AA-pathway (COX and TXA_2-synthase) of *N*′-[(4′-bromo-phenyl)methylene)]-1-(*p*-chlorophenyl)-1*H*-1,2,3-triazole-4-carbohydrazide (**1**) are boxed.

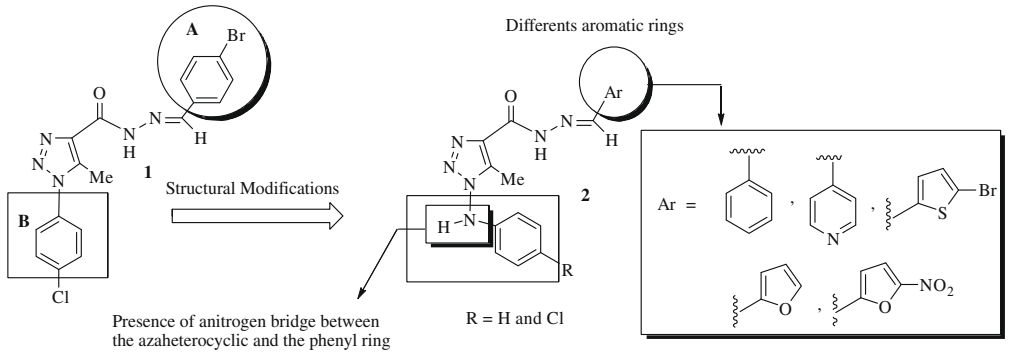


Figure 2. Design of N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides (**2a–j**).

A) of **1** was replaced by a different aromatic ring (e.g., pyridyl, bromothienyl, furyl and nitrofuryl) moiety (Fig. 2). We also modified the nature of the subunit **B** to investigate among other features the contribution of the spacer N–H between the azaheterocyclic for the biological activity of this new series of compounds. Finally, these derivatives were submitted to an in silico oral biodisponibility screening, and drugscore and druglikeness evaluation to analyze their overall potential to qualify for a drug, also comparing them to some current antiplatelets drugs (i.e., acetylsalicylic acid, clopidogrel, cilostazol and tirofiban).

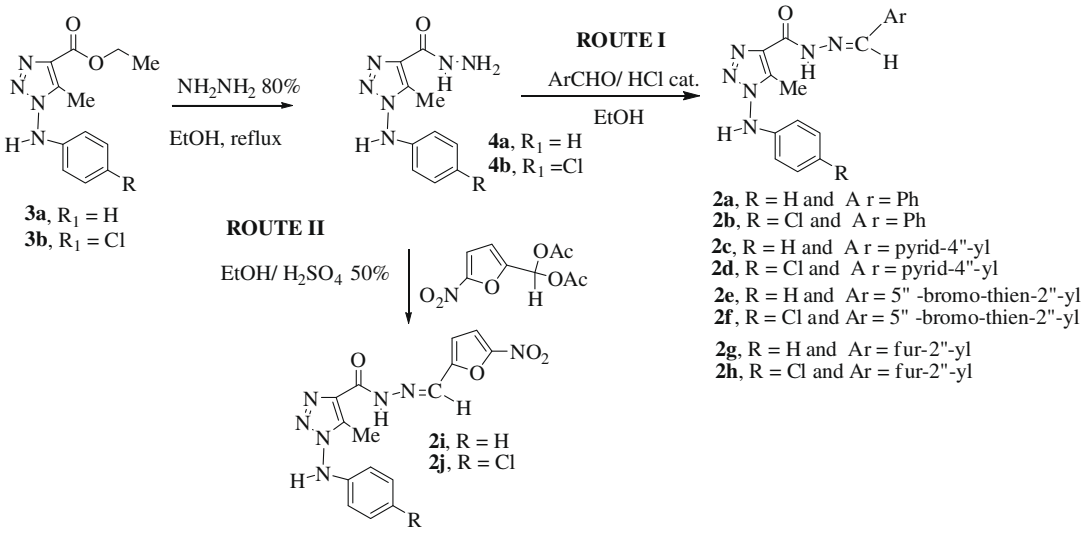
2. Results and discussion

2.1. Chemistry

The synthesis of new 4-acylhydrazone derivatives **2a–j** is shown in Scheme 1. The ethyl N-substituted-phenylamino-4-carbethoxy-1*H*-1,2,3-triazoles **3a–b** were prepared in moderated yields by the condensation of ethyl 2-diazoacetoacetate with substituted phenylhydrazine hydrochlorides, according to our previous report.[15] These compounds were easily converted into the corresponding carbohydrazides **4a–b**, on treatment with hydrazine hydrate in refluxing ethanol[15] (Scheme 1 and Table 1).

The *N*-acylheteroarylhydrazones (*NAH*) **2a–h** were prepared by the condensation of the compounds **4a–b** with the suitable aromatic aldehydes (Route I) in ethanol using hydrochloric as catalyst (Route I), while the 5-nitrofuran derivatives **2i–j** were obtained by the condensation of the corresponding compounds **4a–b** with commercially available 5-nitro-2-furfurylidene diacetate in a mixture of ethanol/sulfuric acid 50% (Route II). The yields of **2a–j** are listed in Table 1.

The *E* and *Z* adducts of the reaction of hydrazine derivatives **4a–b** with aldehydes were in equilibrium with the corresponding hydrazones **2a–j**, through the intermediate hemiaminals **5a–j** (Scheme 2). To assure the diastereomeric ratio of the *NAH* derivatives **2**, which



Scheme 1. Synthetic pathways used for **2a**–**j**.

Table 1
Yields and mps for *NAH* derivatives **2a–j**

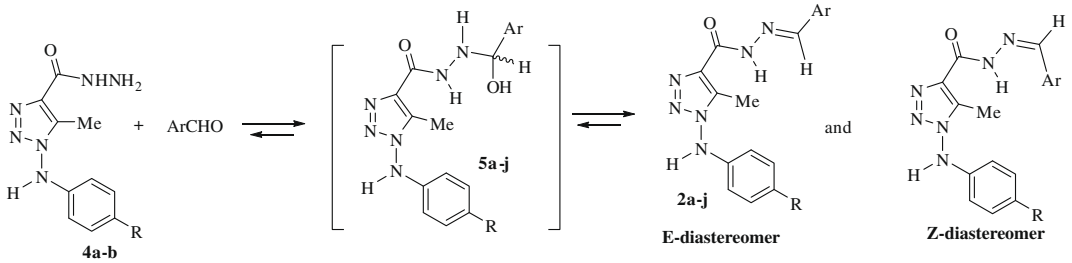
Compound	R	Ar	Mp (°C)	Yield (%)
2a	H	Phenyl	200–202	50
2b	Cl	Phenyl	250–252	67
2c	H	Pyrid-4″-yl	115–125	94
2d	Cl	Pyrid-4″-yl	140–145	70
2e	H	5″-Bromo-thien-2″-yl	100–103	77
2f	Cl	5″-Bromo-thien-2″-yl	180–182	82
2g	H	Fur-2″-yl	212–214	60
2h	Cl	Fur-2″-yl	165–166	85
2i	H	5″-Nitrofur- 2″-yl	175–176	89
2j	Cl	5″-Nitrofur- 2″-yl	235–237	87

was essential for the analysis of the biological results, we determined the stereochemistry of the imine double bond in this series. A detailed analysis of the ^{1}H NMR spectra of these derivatives **2a–j** allowed us to detect only the presence of one N–H signal, which was attributed to the (*E*)-diastereomer, on the basis of nuclear Overhauser (NOE) experiment. For compound **2i**, the irradiation of N–H hydrogen signal at 10.22 ppm resulted in a NOE-enhancement of N=CH hydrogen signal at 8.54 ppm (14.45%). Despite the possibility of formation of two diastereoisomers we only noticed the (*E*)-isomer that was identified and isolated from the reaction.

2.2. Biological assays and Structure–Activity Relationship (SAR) evaluations

To confirm the potentiality of the 4-acylhydrazone moiety for designing new antiplatelet agents, the *NAH* derivatives (**2a–j)** were initially screened at 100 μg ml^{-1} for inhibitory effects on human platelets aggregation using arachidonic acid as agonist (Fig. 3). Interestingly, almost all compounds presented a significant inhibitory profile (⩾50%) at this concentration, except for **2a**, **2g** and **2i.** Thus, these data suggest the maintenance of an antiplatelet profile in these derivatives molecular structure (Fig. 3).

Then we determined the experimental half-maximal inhibitory concentration (IC_{50}) of all derivatives to compare with aspirin, the most used antiplatelet drug current on the market. Interestingly the determination of IC_{50} on arachidonic acid-induced platelet aggregation assays showed three different levels of antiplatelet activity that included lower (8.8–9.1 μg ml^{-1}), similar (32.2–35.5 μg ml^{-1}), or higher (111.5–229.9 μg ml^{-1}) values than that of aspirin (32.5 ± 0.2 μg ml^{-1}) (Table 2). The best profile (8.8–9.1 μg ml^{-1}) was observed for compounds **2c**, **2e**, **2f** and **2h**, which infer that the ring moiety substitution seems to be more important than R position to this inhibitory activity since both **2e** and **2f** present different R substituents and same ring moiety (bromothienyl) (Table 2). However R position is also significant to this acid arachidonic-induced platelet aggregation inhibitory activity as this profile may be negatively (i.e., **2d**) or positively affected (i.e., **2b**, **2h** and **2j**) when the ring is replaced with the addition of chlorine at R position compared to **2a** (Table 2). The maintenance of hydrogen at R position and the presence of furyl, phenyl or nitrofuryl moiety generated the less active compounds (**2a**, **2g** and **2i**) against arachidonic acid-induced platelet aggregation (Table 2). However the heterogeneous electronic distribution in the ring moiety caused by the heteroatom presence (i.e., oxygen, sulfur and nitrogen) apparently still favours the interaction of the derivative with the platelet target when compared to a phenyl ring (i.e., **2a**).



Scheme 2. Equilibrium of *E*- and *Z*-hydrazones through intermediate hemiaminals.

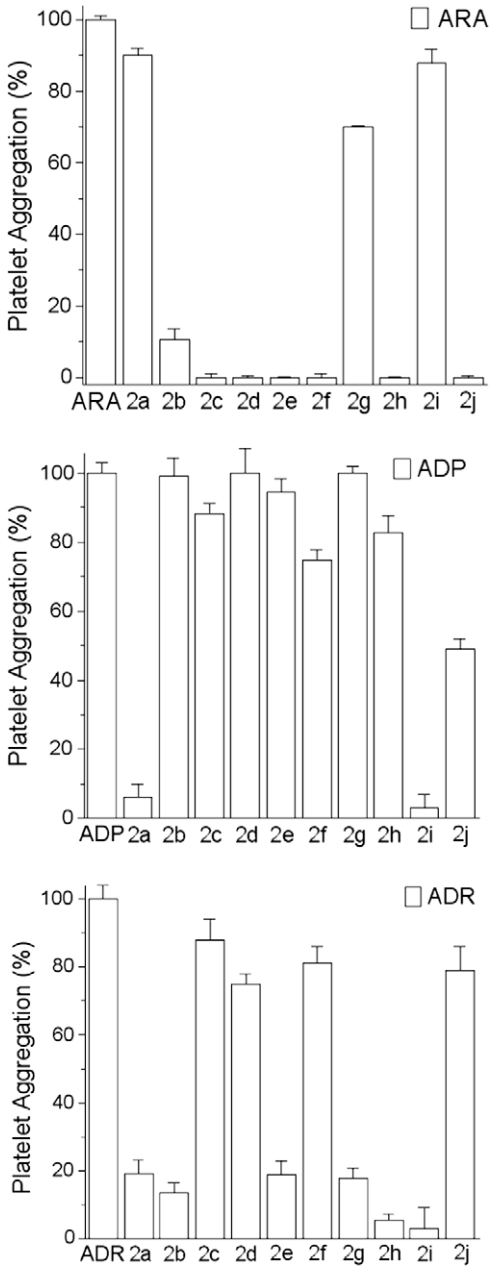


Figure 3. Inhibitory effect (%) of the N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides (**2a–j**) (100 μg ml^{-1}) on in vitro platelet aggregation of human citrated platelet-rich plasma induced by adrenaline (ADR), arachidonic acid (ARC), or adenosine diphosphate (ADP). ADR, ARC and ADP are the positive aggregatory controls without the derivatives.

Given the redundance of discrete pathways leading to platelet aggregation, some known antiplatelet drugs such as aspirin inhibit TXA_2-mediated, adrenaline-mediated and ADP-mediated platelet aggregation, whereas leave the activity of other platelet agonists such as thrombin largely unaffected.[6] Interestingly our evaluation of these derivatives using ADP or adrenaline as agonists showed different results from that observed for aspirin (Table 2).

Surprisingly only **2a** and **2i** showed a significant antiplatelet profile (>50%) against ADP-induced platelet aggregation at the screening concentration (100 μg ml^{-1}) (Fig. 3). In fact most of the *NAH* derivatives presented an IC_{50} significantly higher (136.4–337.5 μg ml^{-1}) than that of aspirin (21.5 ± 2.4 μg ml^{-1}) (Table 2). These results may possibly suggest a different target for these derivatives than that of aspirin (COX_1). Despite of that, the inhibition of common steps such as the integrin αIIbβ3 (GPIIb/IIIa), which is expressed on the platelet surface and a final common pathway of platelet aggregation caused by any agonist[6] is initially discarded since these derivatives present significantly different degree of activity against arachidonic acid and ADP-induced platelet aggregation assays.

The experimental results of adrenaline-induced platelet aggregation assays showed that most of the derivatives were able to significantly inhibit this process at the screening concentration (100 μg ml^{-1}) except for **2c**, **2d**, **2f** and **2j**. However these less active compounds were the most active against the human platelet aggregation induced by arachidonic acid. Once more these data reinforce the hypothesis of a different target, other than that of aspirin. The IC_{50} determination revealed **2a** and **2i** as mixed ADP/adrenaline antagonists whereas **2g** acted as a more specific adrenaline antagonist (Table 2). The structure–activity analysis of the derivatives structure suggested that the chemical requirements for establishing a significant inhibitory activity against adrenaline-induced platelet aggregation are different than those apparently required for other agonists (i.e., arachidonic acid). Apparently a molecule with a smaller or less polar ring with no substitution at R position is preferred for displaying a higher inhibitory activity (IC_{50} < 10 μg ml^{-1}), except for **2h**.

2.3. In silico oral biodisponibility—molecular modeling approach

The N-substituted-phenylamino-5-methyl-*1H*-1,2,3-triazole-4-carbohydrazides (**2a–j)** were submitted to an in silico evaluation using a molecular modeling approach. Since a good absorption after oral administration is obligatory for antiplatelet medical use purpose, we analyzed these derivatives according to the rule-of-five developed by Lipinski et al. (Table 2).[20] The rule-of-five theoretically indicates if a chemical compound could be an orally active drug in humans. The rule states that the most 'druglike' molecules present clog$P \leqslant 5$, molecular weight (MW) $\leqslant 500$, and number of hydrogen bond acceptors $\leqslant 10$ and donors $\leqslant 5$. Molecules violating more than one of these rules may have problems with bioavailability. The results showed that all compounds of the N-substituted-phenylamino-5-methyl-*1H*-1,2,3-triazole-4-carbohydrazides (**2a–j)** fulfilled the Lipinski 'rule-of-five' (Table 2). Importantly, according to the theoretical analysis of the lipophilicity (clog*P*), the most active inhibitors (IC_{50} < 10 μg ml^{-1}) of arachidonic acid-pathway (**2c**, **2e**, **2f** and **2h**), ADP-pathway (**2a** and **2i**) or adrenaline-pathway (**2a**, **2e**, **2h** and **2i**) were sufficiently hydrophobic for penetrating the biological membranes in Lipinski rules, except for **2f** and **2i**.

Herein, we also calculated the druglikeness and drugscore values for these derivatives to analyze their overall potential to qualify for a drug including the comparison with some drugs currently in use in therapy against atherothrombotic disease (i.e., ASA, clopidogrel, cilostazol and tirofiban) (Fig. 4). The druglikeness value is calculated based on the occurrence frequency of every one of the fragments of the analyzed molecules and compared to commercial drugs and non-druglike compounds. In the Osiris program, the occurrence frequency of each fragment is determined within the collection created by shredering 3300 traded drugs as well as 15,000 commercially available chemicals (Fluka) yielding a complete list of all available fragments. In this case, positive values point out that the molecule contains predominantly the better fragments, which are frequently present in commercial drugs but not in the non-druglike collection of Fluka compounds. The drugscore combines druglikeness, clog*P*, log*S*, molecular weight and toxicity risks in one handy value that may be used to judge the drug potential of a compound. Interestingly we noticed the compounds with a positive druglikeness (1.27–5.01) and drugscore

Table 2
Comparison of biological and theoretical features of *NAH* derivatives **2a–j** including experimental half-maximal inhibitory concentration (IC_{50} in $\mu g\ ml^{-1}$) on in vitro human platelet aggregation assays using arachidonic acid, adenosine diphosphate (ADP) and adrenaline as agonist and theoretical oral biodisponibility (Lipinski rule-of-five) predicted by using a molecular modeling approach

Compound	R	Ar	Experimental IC_{50} ($\mu g\ ml^{-1}$)			Theoretical oral biodisponibility (Lipinski rule-of-five)[a]			
			Arachidonic acid	ADP	Adrenaline	HBA	HBD	Molecular weight	cLog*P*
2a	H	Phenyl	229.9 ± 19.8	19.04 ± 3.3	9.6 ± 0.25	7	2	320.35	4.07
2b	Cl	Phenyl	32.2 ± 2.1	180.4 ± 4.3	28.9 ± 4.2	7	2	354.80	4.69
2c	H	Pyrid-4″-yl	8.8 ± 0.1	277.8 ± 54.5	161.1 ± 5.5	8	2	321.34	2.99
2d	Cl	Pyrid-4″-yl	33.1 ± 0.8	168.6 ± 5.4	144.6 ± 1.9	8	2	355.79	3.61
2e	H	5″-Bromo-thien-2″-yl	9.1 ± 0.2	316.9 ± 59.9	13.4 ± 0.5	7	2	405.28	4.88
2f	Cl	5″-Bromo-thien-2″-yl	9.1 ± 0.1	332.5 ± 6.6	144.2 ± 8.8	7	2	439.72	5.49
2g	H	Fur-2″-yl	111.5 ± 1.7	337.5 ± 35.7	20.1 ± 4.1	8	2	310.31	3.17
2h	Cl	Fur-2″-yl	8.9 ± 0.1	261.0 ± 22.3	7.8 ± 0.7	8	2	344.76	3.79
2i	H	5″-Nitrofur- 2″-yl	169 ± 18.8	11.59 ± 5.9	8.9 ± 0.66	11	2	355.31	3.52
2j	Cl	5″-Nitrofur- 2″-yl	35.5 ± 0.5	136.4 ± 4.3	146.9 ± 1.4	11	2	389.75	4.14
ASA	—	—	32.5 ± 0.2	21.5 ± 2.4	11.7 ± 0.5	4	1	180.16	1.43

Acetylsalicylic acid (ASA) also known as aspirin was used as an antiplatelet control.
[a] For good theoretical oral biodisponibility - number of hydrogen bond acceptors (HBA) ⩽ 10 and donors (HBD) ⩽ 5, clog*P* ⩽ 5 and molecular weight ⩽ 500.

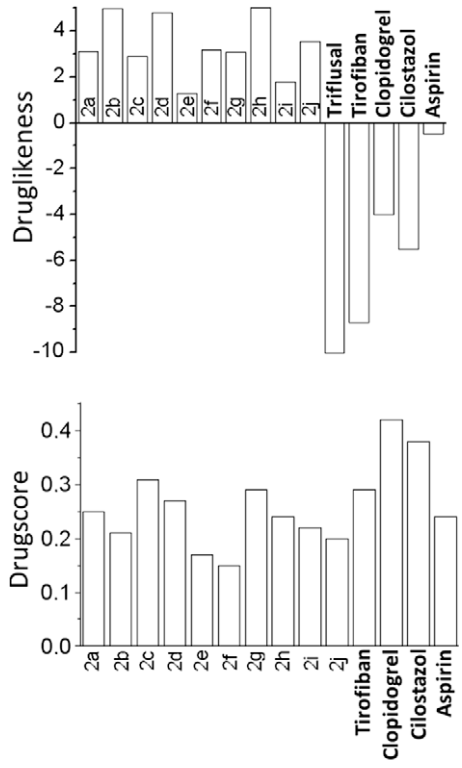


Figure 4. Comparison of the theoretical druglikeness and drugscore values of the N-substituted-phenylamino-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazides (**2a–j**) with current antiplatelet drugs on the market or described in the literature. Positive values are expected for more safe and active molecules.

(0.15–0.31) values were similar or even better than some of the drugs currently used on the market (Fig. 4). The theoretical studies also pointed these derivatives as free of irritant and antireproductive effects (not shown). Although the Osiris risk alerts are not a fully reliable toxicity prediction, the theoretical low-toxicity profile of these compounds reinforces further synthetic and biological exploration for the development of new antiplatelet drugs.

3. Conclusion

In summary, based on our biological and theoretical results we identified some of the synthesized 1,2,3-triazole *NAH* derivatives as potential lead compounds and significant inhibitors ($IC_{50} < 20\ \mu g\ ml^{-1}$) of arachidonic acid, ADP and/or adrenaline-pathways (**2a**, **2c**, **2e**, **2g** and **2h**) to be further in vitro and in vivo investigated. Interestingly, as the antiplatelet profile of these compounds was different from aspirin and compound **1** it suggests other platelet targets for these derivatives than cyclooxygenase.

4. Experimental

Chemical reagents and all solvents used in this study were purshed from Merck AG (Darmstadt, Germany) and VETEC LTDA (Rio de Janeiro, Brazil). Melting points were determined with a Fisher-Johns instrument and are uncorrected. Infrared (IR) spectra were recorded on Perkin–Elmer FT-IR, model 1600 senes spectrophotometer in KBr pellets (Ontario, Canada). NMR spectra, unless otherwise stated, were obtained in deuterated Me_2SO-d_6 using a Varian Unity Plus 300 MHz spectrometer (Palo Alto, California, US). Chemical shifts (δ) are expressed in ppm and the coupling constants (*J*) in hertz. The progress of all reactions was monitored by TLC performed on 2.0 cm × 6.0 cm aluminium sheets precoated with Silica Gel 60 (HF-254, E. Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light at 254 nm. E. Merck silica gel (60–200 mesh) was used for column chromatography. Microanalyses were performed using a Perkin–Elmer Model 2400 instrument and all values were within ±0.4% of the calculated compositions.

The 4-carbethoxy triazoles **3a–b** were prepared by a known procedure described in the literature.[15]

4.1. General procedure for the preparation of the N-substituted-phenylamino-5-methyl-*1H*-1,2,3-triazole-4-carbohydrazides 2a–h

To a solution of hydrazide derivatives **4a–b** (0.50 mmol) in 15 ml of EtOH, was added an equimolar amount of the appropriate aromatic aldehydes in the presence of catalytic amount of aqueous HCl (37%). The reaction was stirred for 2 h, at room temperature. Then, the solvent was evaporated and the precipitate was collected by filtration, washed with cold water and dried under vacuum. The *NAH* derivates **2a–h** were purified in flash chromatography column using 50% *n*-hexane/EtOAc as mixture eluent.

4.1.1. 5-Methyl-*N′*-(phenylmethylene)-1-(phenylamino)-1*H*-1,2,3-triazole-4-carbohydrazide 2a

IR (KBr) ν_{max} (cm^{-1}) 3226 and 3033 (N–H), 1675 (C=O), 1602 (C=N); ^1H NMR (300.00 MHz, DMSO-d_6) δ 2.47 (s, 3H, CH_3), 6.48–6.51 (m, 2H), 6.91–6.96 (m, 1H), 7.24–7.29 (m, 2H), 7.45–

7.47 (m, 3H), 7.71–7.73 (m, 2H), 8.58 (s, 1H, N=C*H*), 10.23 (br s, 1H, N–H), 12.15 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (*C*H$_3$), 112.9 (C-2′ and C-6′), 121.4 (C-4′), 127.1 (C-3″ and C-5″), 128.8 (C-2″ and C-6″), 129.4 (C-3′ and C-5′), 130.0 (C-4″), 134.4 (C-1″), 136.2 (C-4 or C-5), 138.4 (C-4 or C-5), 146.1 (C-1′), 148.1 (N=*C*H), 157.1 (C=O) ppm. Anal. Calcd for $C_{17}H_{16}N_6O$: C, 63.74; H, 5.03; N, 26.23. Found: C, 63.20; H, 5.00; N, 26.10.

4.1.2. 1-(4-Chlorophenylamino)-5-methyl-*N*′-(Phenylmethylene)-1*H*-[1,2,3]-triazole-4-carbohydrazide 2b

IR (KBr) ν_{max} (cm^{-1}) 3292 and 3025 (N–H), 1641 (C=O), 1608 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.47 (s, 3H, C*H*$_3$), 6.53 (d, 2H, *J* = 8.8), 7.3 (d, 2H, *J* = 8.8), 7.45–7.47 (m, 3H), 7.70–7.73 (m, 2H), 8.58 (s, 1H, N=C*H*), 10.36 (br s, 1H, N–H), 12.10 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (*C*H$_3$), 114.6 (C-2′ and C-6′), 125.1 (C-4′), 127.1 (C-3″ and C-5″), 128.8 (C-2″ and C-6″), 129.3 (C-3′ and C-5′), 130.0 (C-4″), 134.3 (C-1″), 136.3 (C-4 or C-5), 138.4 (C-4 or C-5), 145.0 (C-1′), 148.1 (N=*C*H), 156.9 (C=O) ppm. Anal. Calcd for $C_{17}H_{15}ClN_6O$: C, 57.55; H, 4.26; N, 23.69. Found: C, 57.29; H, 4.63; N, 22.94.

4.1.3. 5-Methyl-1-(phenylamino)-*N*′-[(pyrid-4″-yl)methylene]-1*H*-[1,2,3]-triazole-4-carbohydrazide 2c

IR (KBr) ν_{max} (cm^{-1}) 3413 and 3220 (N–H), 1671 (C=O), 1602 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.48 (s, 3H, C*H*$_3$), 6.50–6.53 (m, 2H), 6.91–6.97 (m, 1H), 7.24–7.29 (m, 2H), 7.94 (d, 2H, *J* = 6.2), 8.64 (s, 1H, N=C*H*), 8.79 (d, 2H, *J* = 6.2), 10.30 (br s, 1H, N–H), 12.64 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.2 (*C*H$_3$), 112.9 (C-2′ and C-6′), 121.4 (C-4′), 122.4 (C-2″ and C-6″), 129.4 (C-3′ and C-5′), 135.9 (C-4 or C-5), 139.1 (C-4 or C-5), 145.9 (C-1″), 146.1 (C-1′), 146.1 (C-3″ and C-5″), 146.2 (N=*C*H), 157.0 (C=O) ppm. Anal. Calcd for $C_{17}H_{15}ClN_6O$: C, 63.74; H, 5.03; N, 26.23. Found: C, 63.20; H, 5.00; N, 26.10.

4.1.4. 1-(4-Chlorophenylamino)-5-methyl-*N*′-[(pyrid-4″-yl)methylene]-1*H*-[1,2,3]-triazole-4-carbohydrazide 2d

IR (KBr) ν_{max} (cm^{-1}) 3399 and 3236 (N–H), 1672 (C=O), 1584 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.48 (s, 3H, CH$_3$), 6.55 (d, 2H, *J* = 9.0), 7.31 (d, 2H, *J* = 8.8), 7.94 (d, 2H, *J* = 6.4), 8.64 (s, 1H, N=CH), 8.78 (d, 2H, *J* = 6.4), 10.46 (br s, 1H, N–H), 12.64 (br s, 1H, NH–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (CH$_3$), 114.6 (C-2′ and C-6′), 122.3 (C-2″ and C-6″), 125.1 (C-4′), 129.2 (C-3′ and C-5′), 135.9 (C-4 or C-5), 139.1 (C-4 or C-5), 144.0 (N=CH), 144.9 (C-1′), 145.9 (C-1″), 146.1 (C-3″ and C-5″), 157.0 (C=O) ppm. Anal. Calcd for $C_{16}H_{14}ClN_7O$: C, 54.01; H, 3.97; N, 27.56. Found: C, 54.40; H, 4.00; N, 27.40.

4.1.5. *N*′-[(5″-Bromothien-2″-yl)methylene]-5-methyl-1-(phenylamino)-1*H*-[1,2,3]-triazole-4-carbohydrazide 2e

IR (KBr) ν_{max} (cm^{-1}) 3472 and 3208 (N–H), 1663 (C=O), 1610 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.45 (s, 3H, C*H*$_3$), 6.51–6.54 (m, 2H), 6.91–6.96 (m, 1H), 7.25 (d, 2H, *J* = 3.9), 7.27 (d, 2H, *J* = 3.9), 7.25–7.33 (m, 2H), 8.67 (s, 1H, N=C*H*), 10.35 (br s, 1H, N–H), 12.18 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (*C*H$_3$), 112.9 (C-2′ and C-6′), 114.4 (C-4″), 121.2 (C-4′), 129.4 (C-3′ and C-5′), 131.0 (C-3″), 131.1 (C-2″), 135.8 (C-4 or C-5), 138.2 (C-4 or C-5), 140.8 (C-1″), 141.9 (N=CH), 145.8 (C-1′), 157.0 (C=O) ppm. Anal. Calcd for $C_{15}H_{13}BrN_6OS$: C, 44.45; H, 3.23; N, 20.74. Found: C, 44.28; H, 3.40; N, 20.39.

4.1.6. *N*′-[(5″-Bromothien-2″-yl)methylene]-1-(4-chlorophenylamino)-5-methyl-1*H*-[1,2,3]-triazole-4-carbohydrazide 2f

IR (KBr) ν_{max} (cm^{-1}) 3439 and 3298 (N–H), 1642 (C=O), 1602 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.43 (s, 3H, CH$_3$), 6.50 (d, 2H, *J* = 9.0), 7.24 (d, 2H, *J* = 3.9), 7.26(d, 2H, *J* = 3.9), 7.29 (d, 2H, *J* = 9.0), 8.62 (s, 1H, N=CH), 10.36 (br s, 1H, N–H), 12.16 (br s, 1H, NH–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.3 (CH$_3$), 114.8 (C-2′ and C-6′), 114.9 (C-4″), 125.4 (C-4′), 129.5 (C-3′ and C-5′), 131.5 (C-3″), 131.6 (C-2″), 136.3 (C-4 or C-5), 138.8 (C-4 or C-5), 141.1 (C-1″), 142.6 (N=CH), 145.1 (C-1′), 157.1 (C=O) ppm. Anal. Calcd for $C_{15}H_{12}BrClN_6OS$: C, 40.97; H, 2.75; N, 19.11. Found: C, 41.39; H, 3.01; N, 19.02.

4.1.7. *N*′-[(Fur-2″-yl)methylene]-5-methyl 1-(phenylamino)-1*H*-[1,2,3]-triazole-4-carbohydrazide 2g

IR (KBr) ν_{max} (cm^{-1}) 3489 and 3204 (N–H), 1663 (C=O), 1610 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.46 (s, 3H, C*H*$_3$), 6.49–6.51 (m, 2H), 6.63 (dd, 1H, *J* = 3.2; 1.9), 6.90 (d, 1H, *J* = 3.4), 6.94–6.96 (m, 1H), 7.23–7.29 (m, 2H), 7.84 (d, 1H, *J* = 1.5), 8.47 (s, 1H, N=C*H*), 10.19 (br s, 1H, N–H), 12.10 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (*C*H$_3$), 112.2 (C-3″), 112.9 (C-2′ and C-6′), 113.3 (C-2″), 121.4 (C-4′), 129.4 (C-3′ and C-5′), 136.2 (C-4 or C-5), 145.1 (C-4″), 138.4 (C-4 or C-5), 137.8 (N=*C*H), 146.1 (C-1′), 149.5 (C-1″), 156.9 (C=O) ppm. Anal. Calcd for $C_{15}H_{14}N_6O_2$: C, 58.06; H, 4.55; N, 27.08. Found: C, 58.05; H, 4.32; N, 27.28.

4.1.8. 1-(4-Chlorophenylamino)-*N*′-[(fur-2″-yl)methylene]-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide 2h

IR (KBr) ν_{max} (cm^{-1}) 3437 and 3222 (N–H), 1672 (C=O), 1592 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.46 (s, 3H, C*H*$_3$), 6.53 (d, 2H, *J* = 8.8), 6.63 (dd, 1H, *J* = 3.4; 1.9), 6.89 (d, 1H, *J* = 3.4), 7.29 (d, 2H, *J* = 8.8), 7.83 (d, 1H, *J* = 1.2), 8.47 (s, 1H, N=C*H*), 10.35 (br s, 1H, N–H), 12.11 (br s, 1H, N*H*–N) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.0 (*C*H$_3$), 112.1 (C-3″), 113.2 (C-2″), 114.6 (C-2′ and C-6′), 125.1 (C-4′), 129.2 (C-3′ and C-5′), 136.2 (C-4 or C-5), 137.7 (N=*C*H), 138.3 (C-4 or C-5), 144.9 (C-1′), 145.0 (C-4″), 149.5 (C-1″), 156.8 (C=O) ppm. Anal. Calcd for $C_{15}H_{13}ClN_6O_2$: C, 52.26; H, 3.80; N, 24.38. Found: C, 51.87; H, 3.57; N, 24.09.

4.2. General procedure for the preparation of the N-substituted-phenylamino-5-methyl-*1H*-1,2,3-triazole-4-carbohydrazides 2i–j

A solution of 5-nitro-2-furfurylidene diacetate (0.4 mmol) in a mixture of EtOH (10.0 ml) with a 50% aqueous solution of sulfuric acid (1.0 ml) was heated for 1–2 min on a steam bath and chilled to the room temperature, and then 0.4 mmol of the **4a–b** were added. The resulting mixture was stirred for 2 h at room temperature and then was poured into crushed ice. The insoluble product was filtered off and purified by column chromatography using 50% *n*-hexane/EtOAc as mixture eluent.

4.2.1. *N*′-[(5″-Nitrofur-2″-yl)methylene]-1-(phenylamino)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide 2i

IR (KBr) ν_{max} (cm^{-1}) 3300 and 3147 (N–H), 1660 (C=O), 1601 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.48 (s, 3H, C*H*$_3$), 6.50–6.52 (m, 2H), 6.92–6.96 (m, 1H), 7.26–7.29 (m, 2H), 7.25 (d, 1H, *J* = 3.9), 7.78 (d, 1H, *J* = 3.9), 8.54 (s, 1H, N=C*H*), 10.22 (br s, 1H, N–H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.1 (*C*H$_3$), 112.9 (C-2′ and C-6′), 114.7 (C-2″), 115.1 (C-3″), 121.4 (C-4′), 129.4 (C-3′ and C-5′), 135.7 (N=*C*H), 135.8 (C-4 or C-5), 139.0 (C-4 or C-5), 146.0 (C-1′), 151.8 (C-1″), 151.8 (C-4″), 157.2 (C=O) ppm. Anal. Calcd for $C_{15}H_{13}N_7O_4$: C, 50.71; H, 3.69; N, 27.59. Found: C, 50.10; H, 3.50; N, 27.30.

4.2.2. *N*′-[(5″-Nitrofur-2″-yl)methylene]-1-(4-chlorophenylamino)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide 2j

IR (KBr) ν_{max} (cm^{-1}) 3273 and 3100 (N–H), 1639 (C=O), 1590 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6) δ 2.47 (s, 3H, C*H*$_3$), 6.54 (d, 2H, *J* = 8.8), 7.25 (d, 1H, *J* = 3.9), 7.31 (d, 2H, *J* = 9.0), 7.78 (d,

2H, J = 3.9), 8.53 (s, 1H, N=C*H*), 10.38 (br s, 1H, N–H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.2 (CH_3), 114.6 (C-2′ and C-6′), 114.7 (C-2″), 115.1 (C-3″), 125.2 (C-4′), 129.3 (C-3′ and C-5′), 135.8 (N=CH), 135.9 (C-4 or C-5), 139.1 (C-4 or C-5), 144.9 (C-1′), 151.8 (C-1″), 151.9 (C-4″), 157.2 (C=O) ppm. Anal. Calcd for $C_{15}H_{12}ClN_7O_4$: C, 46.22; H, 3.10; N, 25.16. Found: C, 46.00; H, 3.20; N, 25.20.

4.3. Platelet aggregation assays

Platelet aggregation was monitored by measuring light transmission according Born method[21] using a aggregometer (Qualiterm, PA-04). Firstly, human platelet-rich plasma (PRP) was prepared from whole blood centrifugation at 120*g* for 5 min at room temperature, and the platelet count was adjusted to $3.0 \times 10^5/\mu L$ to standardize the aggregation study, by adding homologous platelet-poor plasma obtained by centrifugation of the blood at 1500*g* for 5 min. Measurement of platelet aggregation was completed within 3 h of blood sampling. For the screening assays, the PRP was preincubated at 37 °C for 2 min with solvent (DMSO, final concentration 1%) or with the *NAH* derivatives (100 μg ml^{-1} or 1–500 μg ml^{-1}) before the addition of the platelet agonists. Maximal aggregation was obtained stimulating platelets with ADP (10 μmol l^{-1}), adrenaline (0.3 μmol l^{-1}) or arachidonic acid (10 μmol l^{-1}). The effects of *NAH* derivatives were expressed as inhibitory effect (%) compared with control samples containing the inducer only. DMSO (1%) did not interfere significantly with platelet aggregation.

4.4. In silico oral biodisponibility

The theoretical study of oral biodisponibility (Lipinski rule-of-five) was performed in the molinspiration on-line program (http://www.molinspiration.com). The theoretical oral bioavailability ranking of chemical compounds can be estimated using the Lipinski's 'rule-of-five', since it describes molecular properties important for a drug pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (ADME). The active compound must present at least three of four rules: H-bond donors (HBD) $\leqslant$ 5, H-bond acceptors (HDA) $\leqslant$ 10, molecular mass (MM) $\leqslant$ 500, and the calculated log$P$ (clog$P$) $\leqslant$ 5.[20]

The druglikeness and drugscore were also calculated using Osiris Property Explorer (http://www.organic-chemistry.org). Druglikeness is based on the occurrence frequency of each fragment that is determined within the collection of traded drugs and within the supposedly non-druglike collection of Fluka compounds and the drug-score was related to topological descriptors, fingerprints of molecular druglikeness, structural keys and other properties as clog$P$ and molecular weights.[22]

Acknowledgements

This work was supported by the Brazilian agencies FAPERJ and CNPq. Fellowships granted to UFF, by CAPES, CNPq, CNPq-PIBIC, CNPq-REDOXOMA and FAPERJ are gratefully acknowledged. The authors have declared no conflict of interest.

References and notes

1. Yabusaki, K.; Kokufuta, E. *Langmuir* **2002**, *18*, 39.
2. Son, D.-J.; Cho, M.-R.; Jin, Y.-R.; Kim, S.-Y.; Park, Y.-H.; Lee, S.-H.; Akiba, S.; Sato, T.; Yun, Y.-P. *Prostaglandins, Leukot. Essent. Fatty Acids* **2004**, *71*, 25.
3. Schafer, A. I. *Am. J. Med.* **1996**, *101*, 199.
4. Hankey, G. J.; Eikelboom, J. W. *Lancet* **2006**, *367*, 606.
5. Mousa, S. A. *Drug Discovery Today* **1999**, *4*, 552.
6. Born, G.; Patrono, C. *Br. J. Pharmacol.* **2006**, *147*, S241.
7. Hata, A. N.; Breyer, R. M. *Pharmacol. Ther.* **2004**, *103*, 147.
8. Patrono, C.; Bachmann, F.; Baigent, C.; Bode, C.; De Caterina, R.; Charbonnier, B.; Fitzgerald, D.; Hirsh, J.; Husted, S.; Kvasnicka, J.; Montalescot, G.; Rodríguez, L. A. G.; Verheugt, F.; Vermylen, J.; Wallentin, L. *Eur. Heart J.* **2004**, *25*, 166.
9. Mason, P. J.; Jacobs, A. K.; Freedman, J. E. *J. Am. Coll. Cardiol.* **2005**, *46*, 986.
10. Patrignani, P. *Thromb. Res.* **2003**, *110*, 281.
11. Zimmermann, N.; Hohlfeld, T. *Thromb. Haemost.* **2008**, *100*, 379.
12. De La Cruz, J. P.; Moreno, A.; Ruiz-Ruiz, M. I.; De La Cuesta, F. S. *Thromb. Res.* **2000**, *97*, 125.
13. World Health Organization (WHO) http://www.who.int/cardiovascular_diseases/en/. Accessed in 2008.
14. Cunha, A. C.; Figueiredo, J. M.; Tributino, J. L. M.; Miranda, A. L. P.; Castro, H. C.; Zingali, R. B.; Fraga, C. A. M.; Souza, M. C. B. V.; Ferreira, V. F.; Barreiro, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2051.
15. Jordão, A.K. Msc. Thesis, Instituto de Química, UFF, Niterói, Brazil, 2007.
16. Lima, L. M.; Frattani, F. S.; Dos Santos, J. L.; Castro, H. C.; Fraga, C. A. M.; Zingali, R. B.; Barreiro, E. J. *Eur. J. Med. Chem.* **2008**, *43*, 348.
17. Fraga, A. G. M.; Rodrigues, C. R.; Miranda, A. L. P.; Barreiro, E. J.; Fraga, C. A. M. *Eur. J. Pharm. Sci.* **2000**, *11*, 285.
18. Barreiro, E. J.; Fraga, C. A. M.; Miranda, A. L. P. *Quim. Nova* **2002**, *25*, 129.
19. (a) Silva, A. G.; Zapata-Sudo, G.; Kummerle, A. E.; Fraga, C. A. M.; Barreiro, E. J.; Sudo, R. T. *Bioorg. Med. Chem.* **2005**, *13*, 3431; (b) Silva, G. A.; Costa, L. M. M.; Brito, F. C. F.; Miranda, A. L. P.; Barreiro, E. J.; Fraga, C. A. M. *Bioorg. Med. Chem.* **2004**, *12*, 3149.
20. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P . J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3.
21. Born, G. V. R. *Nature* **1962**, *194*, 927.
22. Tekto, I. V. *Drug Discovery Today* **2005**, *22*, 1497.