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# Design, synthesis and analgesic properties of novel conformationally-restricted *N*-acylhydrazones (NAH)

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ABSTRACT

A set of six azaheterocycles were designed as conformationnaly-constrained *N*-acylhydrazones and tested as analgesics.



Among the class of *N*-acylhydrazones (NAH) developed at our laboratory, some of them presented various pharmacological properties, specially analgesic and anti-inflammatory.[1] It is well known that medicinal chemistry tools including molecular hybridization,[2] bioisosterism,[3] molecular simplification,[4] homologation or conformational restriction[5] may dramatically change the pharmacological profile of a given molecule and were utilized on the first series of bioactive NAH's. As a prototypical example given in Scheme 1, NAH's **1** may be considered as *seco* derivatives of 6-aryl pyridazinones **2**.

We recently identified two compounds (cpds **3** and **4**) as analgesics with about the same potency (inhibition of AcOH-induced constriction in mice, $ED_{50}$ about 2 mg/kg, see Table 1).

The aim of this work was the design and synthesis of a set of NAH structurally-related compounds, as depicted in Chart 1.

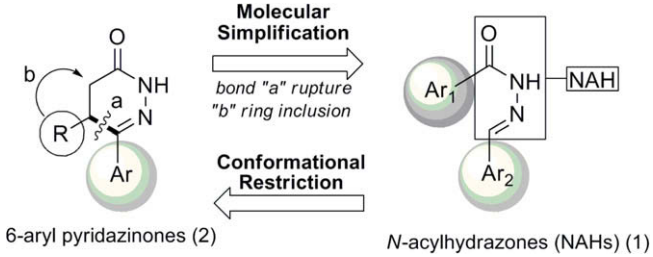
Among the various conformational possibilities provided by the *N*-acylhydrazone chemotype, we selected the most favorable conformations highlighting:

(i) *E* or *Z* relative configuration for the phenyl imine function.
(ii) *E* or *Z* for the benzamide group.
(iii) *s-cis* or *s-trans* junction between both functions.

Finally three different main prototypical geometries could be easily identified, as depicted in Chart 1.

We focused our interest on these conformers (**I**–**III**) for building semi-constrained NAH structural analogues. As illustrated in Chart 1, the full extended semi-folded conformation **I** provided the quinazoline **5** by bridging the *N*-acyl nitrogen onto the *ortho* position of the benzamide (mode **a**), whereas bridging the oxygen amide of **3** in a similar fashion through an amidine isosteres (mode **b**) gave an hydrazinoquinazoline (**6**). Through another type of rigidification (modes **c** and **d**) starting from the conformer **II**, two other heterocycles were identified. The first one results from the incorporation of the hydrazine into a ring system, a pyridazine (**7**), or a pyrazole ring (**8**) (mode **d**).

In the first compound (**7**), another constraint was introduced by means of a triazole ring (mode **c**) leading to a rigid core (triazolo [4,3-*b*] pyridazine) bearing two aromatics.




**Scheme 1.** Structural pattern of NAHs framework included in the piridazinone ring.

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**Table 1**
Antinociceptive activity for derivatives **3**–**10**, paracetamol and indomethacin in the inhibition of AcOH-induced constrictions (0.1 N) in mice

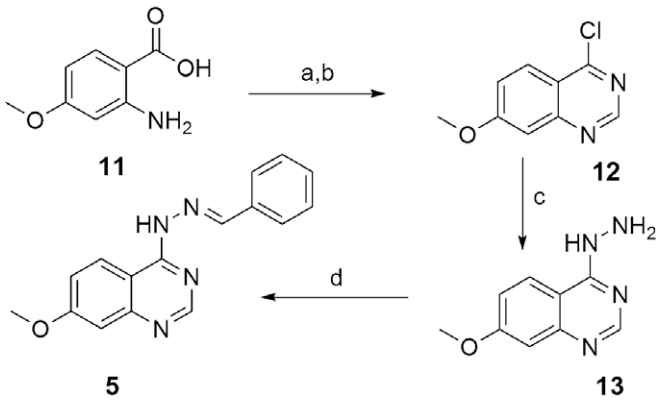
| Compounds | Molecular formula | Mw | Dose | | Number of constrictions[a] | Analgesic activity[b] | $ED_{50}$ | |
|---|---|---|---|---|---|---|---|---|
| | | | μmol/Kg | mg/Kg | | | μmol/Kg | mg/Kg |
| Vehicle (arabic gum) | — | — | — | — | 60.6 ± 1.2 | — | — | — |
| Paracetamol | $C_8H_9NO_2$ | 151 | 100 | 15 | 38.8 ± 0.9 | 36[c] | — | — |
| Indomethacin | $C_{19}H_{16}ClNO_4$ | 358 | 100 | 36 | 35.1 ± 1.3 | 42[c] | 56.2 | 20.1 |
| **3** | $C_{15}H_{14}N_2O_2$ | 254 | 100 | 25 | 28.2 ± 1.5 | 54[c] | 9.2 | 2.3 |
| **4** | $C_{16}H_{16}N_2O_2$ | 268 | 100 | 27 | 30.4 ± 0.7 | 50[c] | 5.9 | 1.6 |
| **5** | $C_{16}H_{14}N_4O$ | 278 | 100 | 28 | 43.0 ± 2.4 | 28[c] | — | — |
| **6** | $C_{16}H_{13}N_3O_2$ | 279 | 100 | 28 | 31.8 ± 2.5 | 48[c] | 4.2 | 1.2 |
| **7** | $C_{18}H_{14}N_4O$ | 302 | 100 | 30 | 47.2 ± 3.1 | 22[c] | — | — |
| **8** | $C_{18}H_{16}N_2O_2$ | 292 | 100 | 29 | 30.4 ± 2.7 | 50[c] | 5.1 | 1.5 |
| **9a** + **9b** | $C_{15}H_{12}N_2O_2$ | 252 | 100 | 25 | 40.6 ± 2.0 | 33[c] | — | — |
| **10** | $C_{16}H_{14}N_2O_2$ | 266 | 100 | 27 | 38.6 ± 1.4 | 36[c] | — | — |

All compounds were administered orally; $N = 7$–10 animals.
[a] Results expressed in terms of mean ± SEM.
[b] Percentage of inhibition obtained by comparison with the vehicle control group.
[c] $p < 0.05$ (ANOVA one way followed by Dunnet test).

By another hand, considering the semi-folded conformer **III**, two other heterocycles were designed. The phtalazinone **9** (direct bridging the imine carbon onto *ortho* position of benzamide, mode **e**) and its homologous 2,3 benzodiazepinone **10** (bridging the imine carbon onto *ortho* position of benzamide through a methylene group) were also selected.

The successive chemical methodologies yielding the compounds **5**–**10** are described, as following.

Quinazoline **5** was prepared according to Scheme 2 starting from the anthranilic acid **11** by a cyclization with formamidine acetate under microwaves heat followed by chlorination of the intermediate quinazolinone with $POCl_3$ to furnish the chloroquinazoline **12**.[6] Reaction of **12** with hydrazine hydrate followed by acid catalyzed condensation of **13** with benzaldehyde generated the desired quinazolinyl-hydrazone derivative.[7] The careful analysis of $^1H$ NMR spectra of **5** demonstrated the presence of only one imino hydrogen signal at $\delta$ 8.4 ppm, similar to the shift observed for the lead compound **3** (see Supplementary data), which was




**Scheme 2.** Reaction conditions: (a) formamidine acetate, EtOH, μW 120 °C, 40 min, 87%; (b) $POCl_3$, 90 °C, 1 h, 97%; (c) $NH_2NH_2{\cdot}H_2O$, 90 °C, 6 h, 88%; (d) benzaldehyde, EtOH, HClcat, 1 h, 92%.

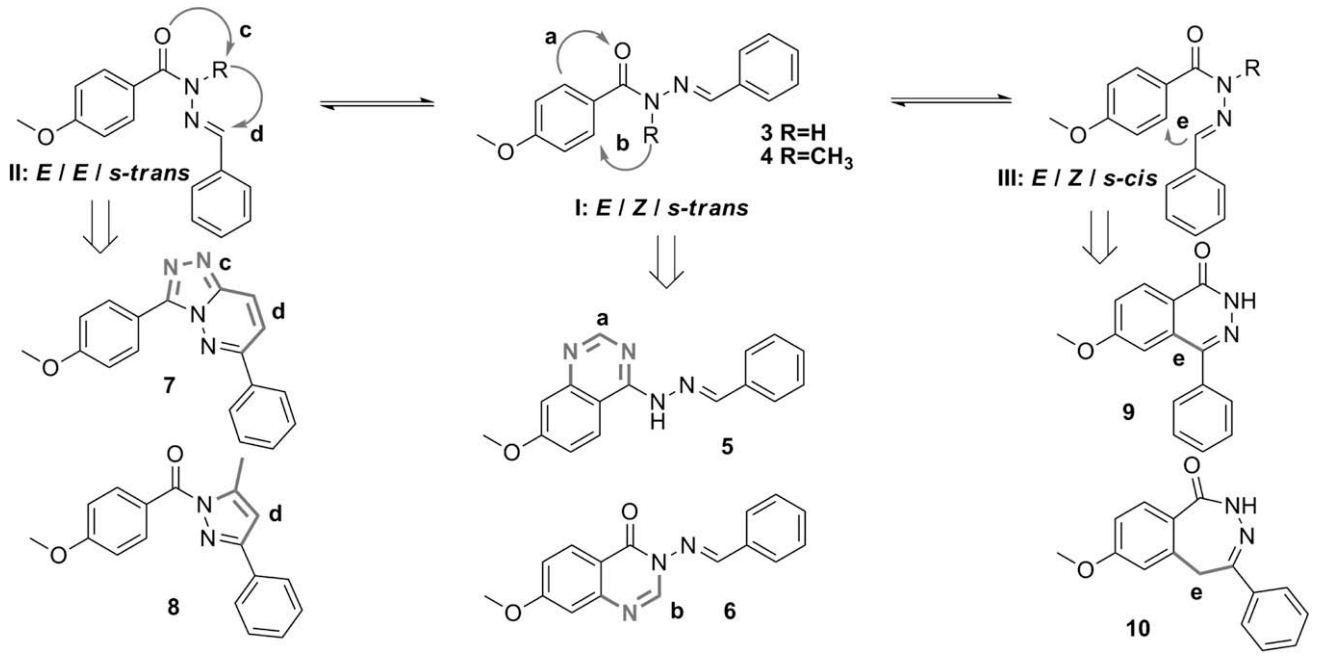
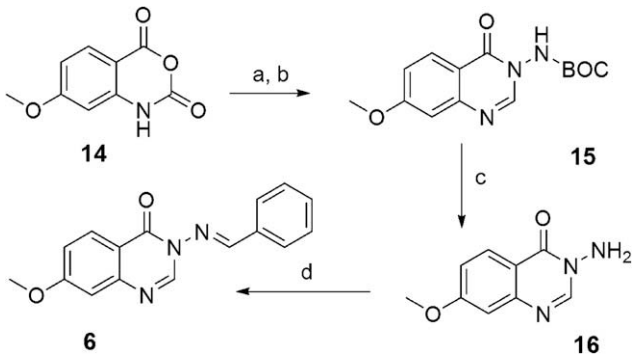
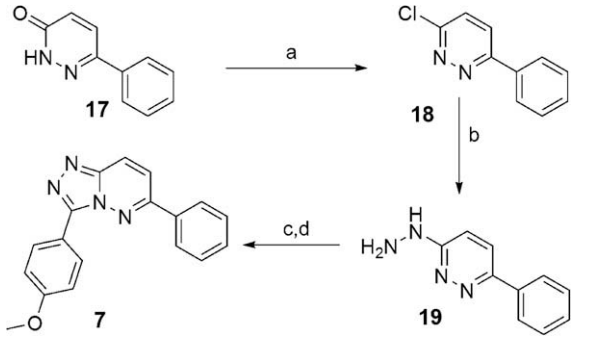



**Chart 1.** Design of conformationally-restricted derivatives of NAH's 3 and 4.




**Scheme 3.** Reaction conditions: (a) BOC–$NH_2NH_2$, $CH_3CN$, 90 °C, 12 h, 92%; (b) ethyl orthoformate, 90 °C, 4 h, 73%; (c) TFA, $CH_2Cl_2$, 1 h, 89%; (d) benzaldehyde, EtOH, HClcat, 1 h, 87%.




**Scheme 4.** Reaction conditions: (a) $POCl_3$, 100 °C, 2 h, 99%; (b) $NH_2NH_2{\cdot}H_2O$, EtOH, 90 °C, 2 h, 90%; (c) benzaldehyde, MeOH, HClcat 50 °C, 2 h, 80%; (d) DIAD, *n*-BuOH, 120 °C, 4 h, 64%.

attributed to the (*E*)-diastereomer on the basis of several previous reports.[1,8,9]

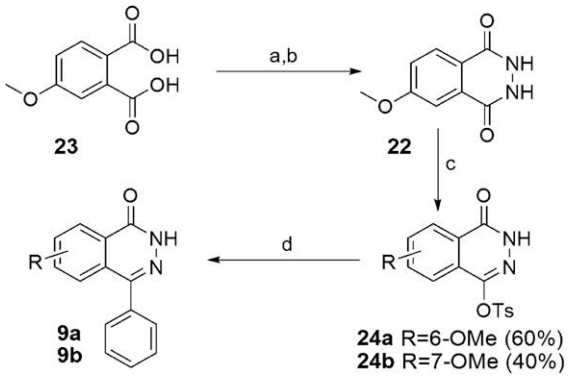
The synthesis of quinazolin-4(3*H*)-one **6** shown in Scheme 3 started from the reaction of *t*-butyl carbazate with isatoic anhydride (**14**)[10] followed by a cyclization with ethyl orthoformate producing the protected quinazolinyl derivative **15**. Deprotection of **15** with TFA followed by acid catalyzed condensation of **16** with benzaldehyde[7] furnished only the desired (*E*)-diastereomer derivative **6**, characterized by the presence of only one imino hydrogen signal in $^1$H NMR ($\delta$ 8.7 ppm).[8]

The 1,2,4-triazolo[4,3-*b*]pyridazine **7** was obtained from the pyridazinone **17** by initial treatment with $POCl_3$, to give the 3-chloropyridazine **18**,[11] followed by a reaction with hydrazine hydrate to yield the hydrazinopyridazine **19** (Scheme 4). This compound (**19**) was then condensed with benzaldehyde in the presence of catalytic amount of acid leading to the corresponding hydrazone. Finally cyclization with DIAD produced the compound **7** in good yield.[12]

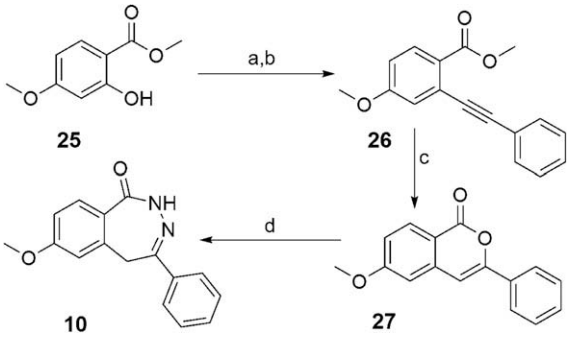
The pyrazole compound **8** was obtained employing the described regioselective N-acylation of 5-methyl-pyrazole **21** with 4-methoxybenzoyl chloride (Scheme 5).[13] This intermediate was obtained from the cyclization of butane-1,3-dione **20** with hydrazine hydrate.

Phtalazinones **9** were prepared as shown in Scheme 6. Condensation of phthalic acid **23** with acetic anhydride followed by addition of hydrazine sulfate led to phtalazine-1,4-dione **22**. Tosylation of **22** produced two regioisomers **24a** and **24b** (ratio 6:4) which were involved in a Suzuki reaction in presence of phenylboronic acid[14] to produce the phthalazin-1(2*H*)-one **9** as a mixture of regioisomers **9a**, **9b** (ratio 6:4 identified by $^1$H NMR signal integration).[11]

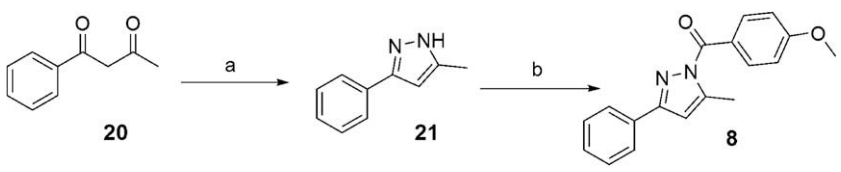
The synthesis of homologous derivative benzo[1,2-*d*]-diazepin-1-one **10** is described in scheme 7. Starting from the commercially available phenol **25**, reaction with triflic anhydride followed by a Sonogashira cross-coupling reaction[15] with phenylacetylene under microwaves irradiation provided the acetylene **26**. Cyclization of **26** in acidic conditions produced the isochromen-1-one **27** which was subsequently reacted with hydrazine hydrate to furnish the final product benzo[1,2-*d*]diazepin-1-one **10**.[16]




**Scheme 6.** Reaction conditions: (a) acetic anhydride, 120 °C, 1 h; (b) $NH_2NH_2{\cdot}H_2SO_4$, DME:$H_2O$, 90 °C, 20 h, 82% two steps; (c) tosyl chloride, pyridine, 80 °C, 6 h, 74%; (d) phenylboronic acid, $Pd(Ph_3)_4$, $Na_2CO_3$, DME:$H_2O$, μW 155 °C, 20 min, 77%.




**Scheme 7.** Reaction conditions: (a) triflic anhydride, $CH_2Cl_2$, $Et_3N$, 0 °C, 1 h, 93%; (b) phenylacetylene, $CH_3CN$, $Et_3N$, $PdCl_2(PPh_3)_2$, $PPh_3$, μW 120 °C, 20 min, 89%; (c) TFA, 6 h, 89%. (d) $NH_2NH_2{\cdot}H_2O$, *n*-BuOH, 90 °C, 6 h, 79%.




**Scheme 5.** Reaction conditions: (a) $NH_2NH_2{\cdot}H_2O$, EtOH, 4 h, 92%; (b) 4-methoxybenzoyl chloride, pyridine, 3 h, 74%.

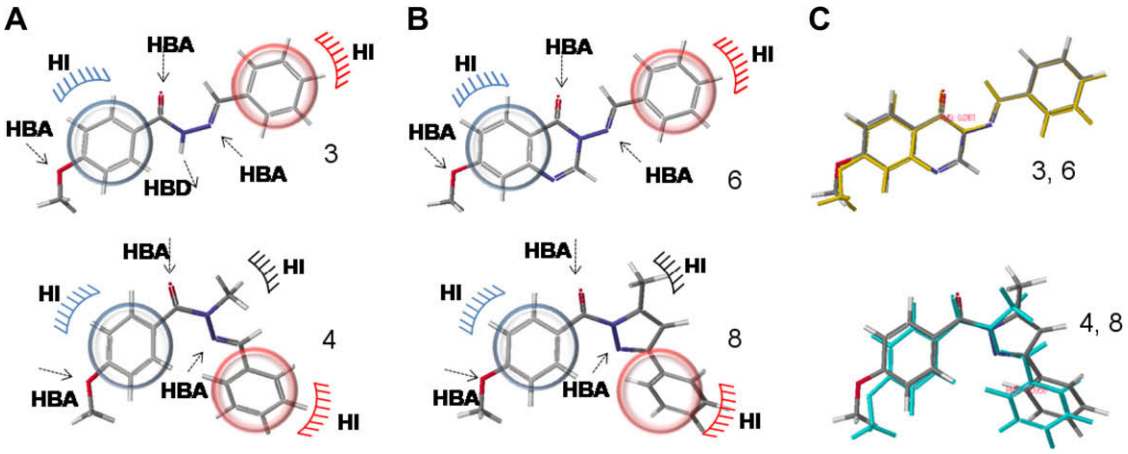



**Figure 1.** Structure–activity relationship between the NAH and cyclic compounds **3**, **4**, **6** and **8**. **HI**—hydrophobic interactions, **HBA**—hydrogen bond acceptor, **HBD**—hydrogen bond donor. (**A**) Putative pharmacophore model for the NAH **3** and *N*-methyl-NAH **4**. (**B**) Compounds **6** and **8** fitted in putative pharmacophore model of original NAHs **3** and **4**. (**C**) Superposition of NAHs **3** (yellow) and **4** (green) and cyclic compounds **6** and **8**.

Among the 6 synthesized heterocycles, that is, **5**, **6**, **7**, **8**, **9a/9b** and **10**, considered as putative **3** and **4** NAH biomimetics, two of them (**6**, **8**) presented similar analgesic properties, in vivo, compared to the NAH lead compounds in the AcOH-induced abdominal constrictions test using oral administrations in mice (Table 1).[17] They were around two times more potent than **3** showing that for these two new derivatives, we were able to improve their bioactivity despite the conformational difference between both classes of compounds. The others four tested compounds (**5**, **7**, **9a/9b** (as a mixture of regioisomers) and **10**) did not present significant activity compared to **3** and **4** in this assay.

To evaluate the similarity of the two new heterocyclic scaffolds (**6**, **8**) with the original lead compounds (**3**, **4**), these compounds were first sketched in the BioMedCAche 6.0 software.[18] Then, the local and global energy minimizations were obtained with semi-empirical AM1 method using dihedral angle search in a 24 steps of 15 degrees by dihedral angle (Fig. 1). The results demonstrated the typically difference between the NAH and the *N*-methyl-NAH,[19] as described for aromatic methyl amides:[20] the N-methylation leads to an amide bond rotation generating a *synperiplanar* conformation, as seen for LASSBio-1385 (**4**).[19] After we have done a putative pharmacophore model for **3** and **4** (Fig. 1A) and we were able to identify that LASSBio-1418 (**8**) and LASSBio-1419 (**6**) fit perfectly in the models made from LASSBio-575 (**3**) and LASSBio-1385 (**4**), respectively. This result validated the initial structural design strategy to exploit the conformational differences between the NAH and *N*-methyl-NAH derivatives (Fig. 1B). In addition, the alignment of the central NAH framework of the lead compounds **3** and **4** with the corresponding heterocyclic scaffolds in **8** and **6** confirmed the correlation between the NAH and the mimic compounds with a RMS ranging from 0.0956 (**4** and **6**) to 0.0161 (**3** and **8**) (Fig. 1C).

The use of conformational restriction in optimization of NAH compounds produced two original analgesic compounds (**6**, **8**) which present scaffolds that have never been reported for this pharmacological activity, and now it can be applied to different NAH derivatives, a structure easily accessed that may present numerous pharmacological profiles.

## Acknowledgments

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.075.

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