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Synthesis and activity studies of analogues of the rat selective toxicant norbormide

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Abstract—Norbormide [5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide] (NRB, **1**), an existing but infrequently used rodenticide, is known to be uniquely toxic to rats but relatively harmless to other rodents and mammals. A series of NRB-related analogues were prepared to investigate the structural features responsible for, and the in vitro biological markers indicative of, in vivo lethality of the parent molecule in rats. Their synthesis and biological evaluation (vasoconstriction, vasodilation, mitochondrial dysfunction, cardiotoxicity and lethality) is described.

1. Introduction

Rats cause substantial damage every year to agricultural interests worldwide. Currently, there are a number of toxicants on the market that are effective in controlling rats, almost all being non-specific broad-spectrum rodenticides. To date, rodent control has been achieved through the use of sub-chronic poisons (e.g., cholecalciferol, bromethalin), acute poisons (e.g., zinc phosphide), first-generation anticoagulants (e.g., warfarin, coumatetralyl, diphacinone, chlorophacinone) and second-generation anticoagulants (e.g., brodifacoum, bromadiolone, bromethalin, difethialone, difenacoum) with varying degrees of success.[1] Second-generation anticoagulants are the most preferred products. Most of these, however, have one common disadvantage in that they have secondary non-target risks associated with them and are dangerous not only to children, but also to domestic pets, wildlife and livestock.

Norbormide, a vasoactive compound 1960s, was introduced to the market as a rodenticide approximately 30 years ago. Found to be uniquely toxic to rats but relatively harmless to other rodents and mammals[2–4] NRB displays unique species-specific constrictor activity that is restricted to the peripheral arteries of the rat. In arteries from all other species tested, as well as in rat aorta and extravascular smooth muscle tissue, NRB exhibits vasorelaxant properties at concentrations that induce vasoconstriction in the rat peripheral arteries.[5] Furthermore, detailed studies conducted upon the individual stereoisomers of NRB, isolated from the *endo* rich stereoisomeric mixture, found the parent compound's physiological effects to be strongly stereospecific. In rat peripheral arteries only the *endo* isomers of NRB retained the contractile activity elicited by the stereoisomeric mixture, with both the *endo* and *exo* isomers exhibiting vasodilatory activity in rat aorta.[6] *In vivo* evaluation established that only the *endo* isomers of NRB were toxic in rats.[7] Also of note is that the stereochemistry at the carbinol carbon (C-5) of NRB has a significant effect on toxicity in that *endo* isomers which bear a *threo* configuration at this centre are 10 times as potent as their *erythro* counterparts.[7]

The mechanisms involved in these divergent effects of NRB have yet to be clarified. Available evidence suggests that the vasoconstrictor effect may be mediated by the stimulation of a number of signal transduction pathways[8] that lead to modulation of calcium influx, presumably mediated by phospholipase C

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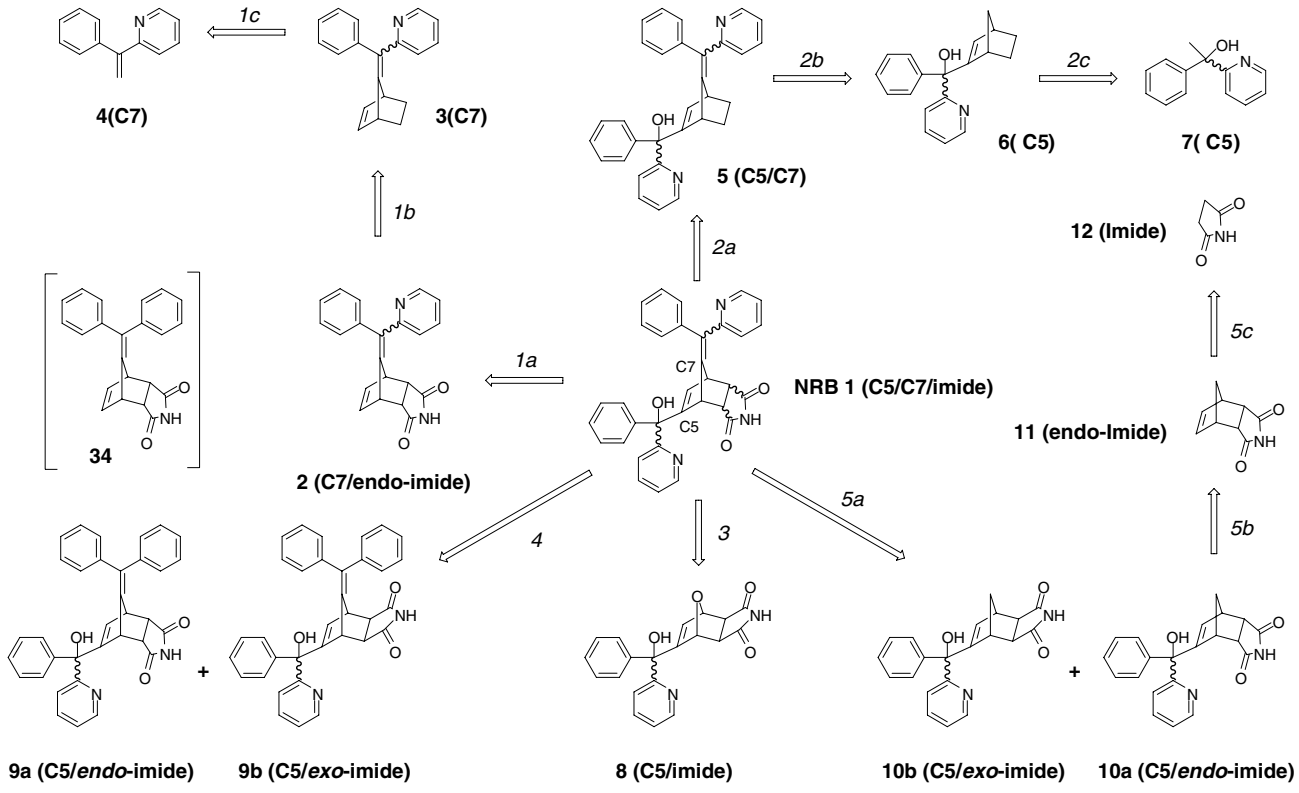


Figure 1. Stepwise 'pseudo-deconstruction' of **NRB** through the synthesis of compounds **2**–**12**.

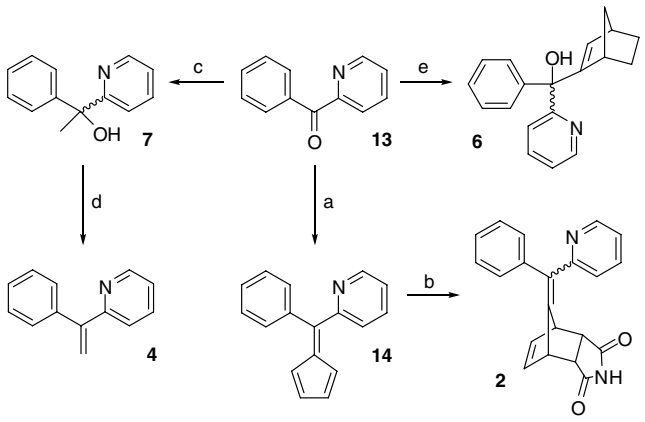
(PLC)-coupled receptors expressed in rat peripheral artery myocytes,[8] whereas the relaxant effect may be the result of a reduction of Ca^{2+} entry through L-type Ca^{2+} channels.[9]

In this paper, we postulate three potential physiological pathways of activity leading to death: (1) selective vasoconstriction of rat small blood vessels, as previous studies indicate that NRB elicits divergent tissue responses, causing selective vasoconstriction of small arteries and vasodilation of large blood vessels in the rat, while dilating both small and large blood vessels of other species;[5] (2) a previously unknown deleterious effect on mitochondrial function which was recently attributed to stimulation of the mitochondrial permeability transition (MPT);[10] and (3) cardiotoxicity/coronaroconstriction as proposed by Yelnosky and Lawlor.[11] Using these in vitro indicators as biological markers for lethality in vivo, a focused analogue design programme[12] was conducted to explore the structural features responsible for each of the individual physiological responses (vasoconstriction, vasodilation, MPT and cardiotoxicity) elicited by NRB.

Herein we report the stepwise 'pseudo-deconstruction' of NRB through the synthesis of a selection of pharmacological probes (**2**–**12**), prepared to establish the influence of the molecule's three key structural features (substituents at C-7, C-5 and the imide unit) on the observed biological activity (Fig. 1). The following modifications were explored: (1a) removal of the group at C-5 from NRB to reveal C-7/*endo*-imide analogue **2**; (1b) further deconstruction of **2** to afford bicyclic C-7 analogue **3**; (1c) simplification of **3** to give C-7 analogue **4**; (2a) removal of the imide ring from NRB to reveal C-5/C-7 analogue **5**; (2b) further deconstruction of **5** to afford bicyclic C-5 analogue **6**; (2c) simplification of **6** to give C-5 analogue **7**; (3) preparation of C-5/imide isostere **8**; (4) replacement of the 2-pyridylbenzylidene unit at C-7 with a phenylbenzylidene group to reveal alternative C-5/imide analogues **9a** and **9b**; (5a) removal of the group at C-7 from NRB to reveal C-5/imide analogues **10a** and **10b**; (5b) further deconstruction of **10a** to afford bicyclic *endo*-imide analogue **11**; (5c) simplification of **11** to give imide analogue **12**.

2. Chemistry

Compound **2**[13] was accessed via the Diels–Alder reaction of fulvene **14**[14] and maleimide, with exclusive isolation of the *endo*[15] adduct (Scheme 1 and Table 1). Fulvene **14** was selectively obtained over the related 2-fulvenylmethanol **15**[14] through careful control of the reaction conditions, via the slow addition of 2-benzoylpyridine (**13**) to an excess of cyclopentadienyl sodium under high dilution, in an effort to limit a second condensation step.[14] Difficulties associated with the preparation of compound **3** focused attention on the synthesis of further simplified C-7 analogue **4**,[16] prepared by the treatment of ketone **13** with methylmagnesium bromide to afford precursor alcohol **7**,[17] with subsequent acid-promoted dehydration[18] providing compound **4** (Scheme 1).



Scheme 1. Reagents and conditions: (a) i—cyclopentadiene, NaOEt, EtOH, rt, 0.5 h, ii—**13**, EtOH, rt, 2.5 h; (b) maleimide, PhH, reflux, 18 h, 25% (two steps); (c) MeMgBr, THF, rt, 18 h, 95%; (d) H_2SO_4 (85% aq), 100 °C, 15 min, 83%; (e) i—norbornene, KO^{t}Bu, ^{n}BuLi, THF, −78 °C, 15 min warming to −40 °C, 1 h, ii—LiBr, THF, −70 °C warming to −50 °C, 30 min, iii—**13**, THF, −70 °C, 30 min then H_2O, 20%.

Table 1. Isomeric ratios of **NRB** and compounds **2**, **5**, **6** and **8**–**10**

Compound	Stereochemistry	Ratio
NRB[a]	*cis-threo*/*trans-threo*/*cis-erythro*/*trans-erythro*	2:2:1:1
2[b]	*cis*/*trans*	1:1
5	*cis-threo*/*trans-threo*/*cis-erythro*/*trans-erythro*	1:1:1:1
6	*erythro*/*threo*	1:1
8[c]	*erythro*	Exclusive
9a[b]	*erythro*/*threo*	15:85
9b[c]	*erythro*	Exclusive
10a[b]	*erythro*/*threo*	1:2
10b[c]	*erythro*/*threo*	55:45

[a] >90% *endo* isomer.
[b] Exclusively *endo* isomer.
[c] Exclusively *exo* isomer.

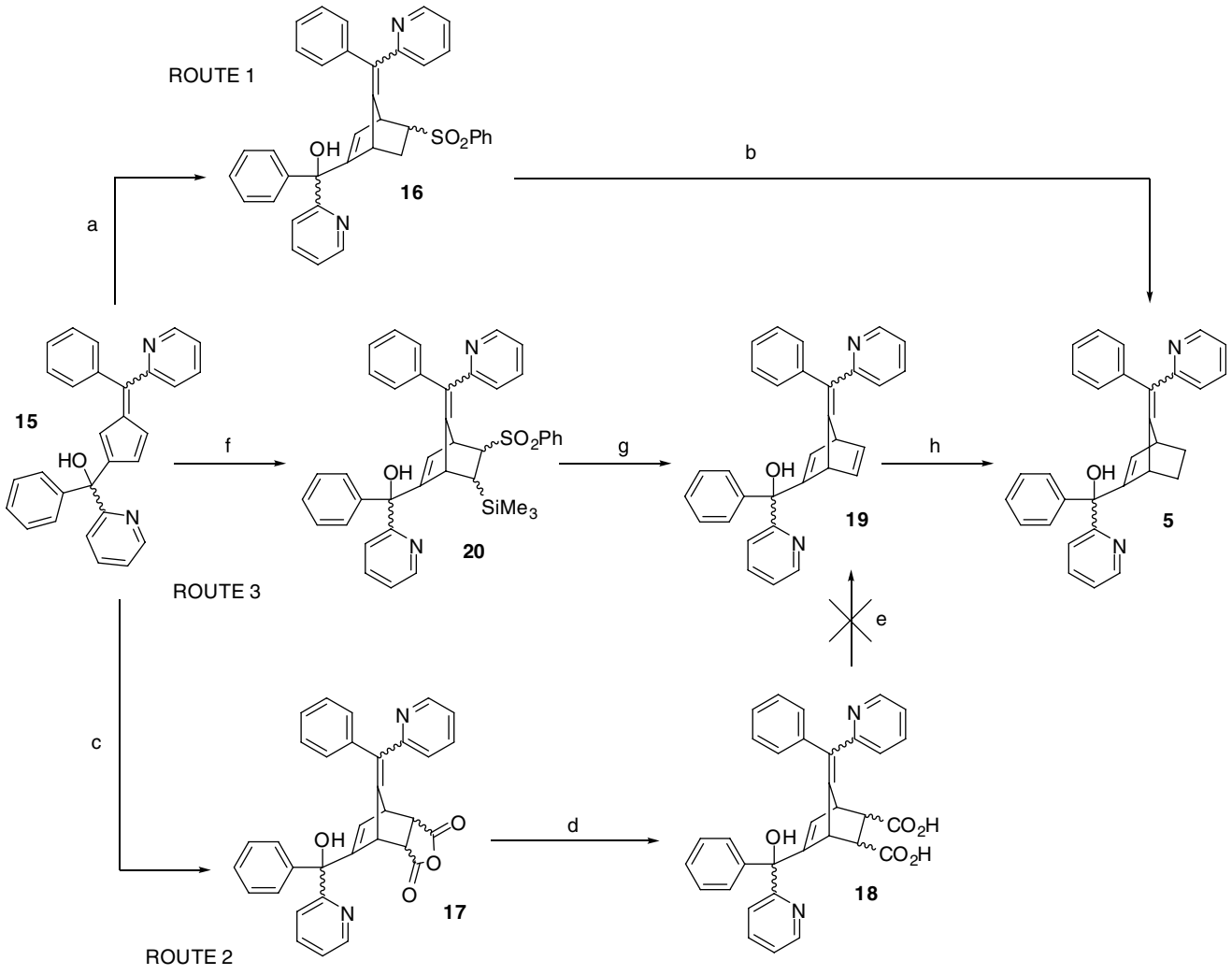
Retrosynthetic analysis revealed **5** to be the cycloaddition product of 2-fulvenylmethanol **15** and ethylene (Scheme 2 and Table 1). Due to the low reactivity of ethylene as a dienophile, together with its explosive nature under pressure, initial efforts focused on accessing **5** via a Diels–Alder reaction of **15** with ethylene equivalent phenyl vinyl sulfone.[19] Subsequent desulfonation[19] of adduct **16**, using sodium-mercury amalgam, successfully afforded **5** albeit in low yield (Scheme 2, Route 1). A second approach to **5** focused on the bis-decarboxylation of vicinal dicarboxylic acid **18**,[20] prepared over two steps from **15** via the hydrolysis of maleic anhydride adduct **17**[21] (Scheme 2, Route 2). Oxidative bis-decarboxylation[22] of **18** using copper (I) oxide in quinoline at elevated temperatures failed to afford olefin precursor **19**. Likewise, both lead tetra-acetate-promoted bis-decarboxylation[23] of **18** and perester decomposition[24] routes to **19** also proved unsuccessful. Ultimately a modified Diels–Alder approach employing acetylene equivalent **21**[25] provided access to **5** in three steps (Scheme 2, Route 3). Subsequent fluoride-induced elimination of β-silylsulfone **20**, using *tetra*-butylammonium fluoride at elevated temperatures, afforded precursor olefin **19**, which underwent regioselective hydrogenation of the disubstituted olefin to afford **5** as a mixture of isomers (Scheme 2 and Table 1).

Analogue **6** was directly accessed in a single step from norbornene using Schlosser's conditions (a mixture of *n*-butyllithium and potassium *tert*-butoxide) to effect formation of the vinyl lithium species,[26] that was subsequently trapped with ketone **13** (Scheme 1 and Table 1).

At this stage it was assumed that compound **10** could not be prepared, due to the synthetic inaccessibility of the appropriate precursor diene **33**. In light of this difficulty an isostere of **10**, namely **8**, was prepared. Compound **8** was accessed via the Diels–Alder reaction of substituted furan **26** and maleimide to afford adducts **8a** and **8b** (*endo*:*exo* 2:98) (Scheme 3 and Table 1). α-Bromoester **23**[27] was prepared in three steps from maleic acid (**22**) via sequential esterification, bromination and dehydrobromination. Reduction[28] of **23** using diisobutylaluminium hydride afforded diol **24**,[28] which underwent oxidative cyclization[29] in the presence of chromic acid (generated in situ) to give 3-bromofuran (**25**).[30] Substituted furan **26** was prepared via reaction of the 3-lithio derivative of **25** with 2-benzoylpyridine (**13**).[31] A more efficient route to **26** proceeded via 3,4-dibromofuran (**29**),[30] prepared in two steps from 2-butyne-1,4-diol (**27**) via sequential dibromination[32] (to give **28**[32]) and oxidative cyclization.[29,33] Subsequent formation of the mono-lithiated furan,[30] using *tert*-butyllithium at low temperature, followed by quenching with water gave 3-bromofuran (**25**), which was used as described previously.

Given that **8** was found to be an unsuitable model for **10a**, due to the *exo*-orientation of the imide ring, compound**9a** was proposed as an alternative C-5/*endo*-imide analogue. This approach was based on the ability of the phenylbenzylidene subunit at C-7 to 'force' the bicyclic system to adopt an *endo*-conformation, and with *endo*-imide compound **34**[34] (Fig. 1) having proven to be inactive within previous studies of this type it was hoped that the phenylbenzylidene group would have little direct influence on the overall pharmacological behaviour of compound **9**. Analogue **9** was accessed via the Diels–Alder reaction of 2-fulvenylmethanol **32**[35] and maleimide to afford adducts **9a** and **9b** (*endo*:*exo* 1.2:1), which were subsequently separated by column chromatography (Scheme 4 and Table 1). 2-Fulvenylmethanol **32** was prepared in two steps through the reaction of benzophenone (**30**) with cyclopentadienylmagnesium bromide (to give **31a** and **31b**), followed by a second condensation step with 2-benzoylpyridine (**13**) and concurrent dehydration to afford **32**.[35,36] Information gathered during the preparation of compound **9**, in particular the synthesis of precursor **31**,[35] led us to believe that the preparation of compound **10**, via diene **33**,should be possible.

Compound **10** was thus accessed in a similar fashion via Diels–Alder reaction of maleimide with substituted cyclopentadiene **33**,[35] to afford adducts **10a** and **10b** (*endo*:*exo* 6:1), which were subsequently separated by



Scheme 2. Reagents and conditions: (a) phenyl vinyl sulfone, xylene, reflux, 18 h, 50%; (b) Na/Hg amalgam, THF/MeOH, rt, 18 h, 5%; (c) maleic anhydride, PhH, reflux, 18 h, 80%; (d) NaOH (1 M aq), H_2O, 60 °C, 18 h, 90%; (e) Cu_2O, quinoline, 2,2′-dipyridyl, powdered glass, 180 °C, 24 h, reaction failed; (f) (*E*)-$Me_3SiCH{=}CHSO_2Ph$ (**21**), PhMe, reflux, 48 h, ca. 22%; (g) TBAF, THF, reflux, 48 h, 20%; (h) H_2 (1 atm), Pd/C, EtOAc, rt, 2 h, 100%.

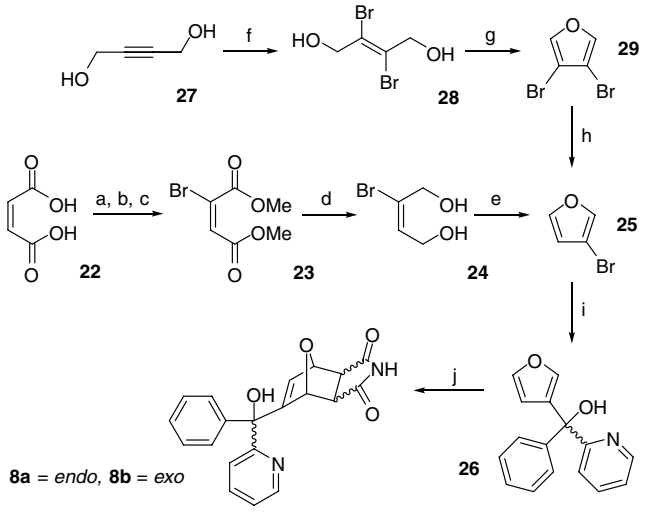
column chromatography (Scheme 5 and Table 1). Compounds **11** and **12** were sourced commercially.

Although the following biological evaluation of compounds **2**–**12** deals primarily with isomeric mixtures (Table 1), the stereochemical makeup of the analogues in the main reflects that of the stereoisomeric NRB mixture to which they are compared.

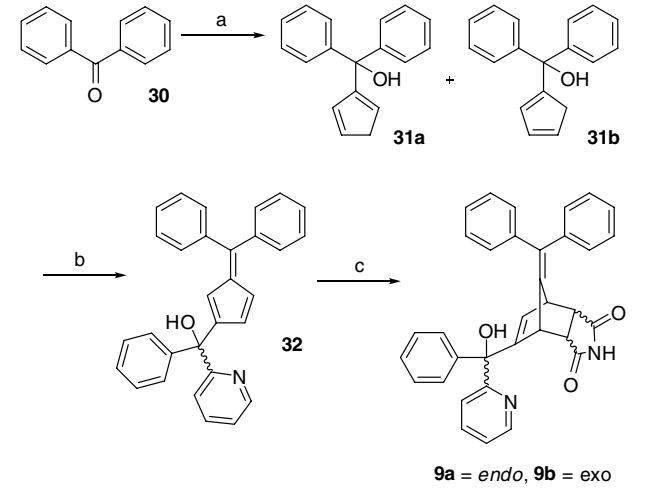
3. Results and discussion

With NRB known to be a constrictor of rat peripheral arteries and a relaxant in rat aorta,[37] initial studies were conducted in an effort to characterize the corresponding analogues for their contractile and relaxing effects on rat caudal artery and aortic vascular smooth muscle, respectively. As depicted in Figure 2, of the compounds prepared, only analogues **2**, **4** and **9a** retained the contractile activity elicited by NRB. While these compounds were found to be considerably less potent than NRB, as reflected by their higher EC_{50} values (Table 2), in terms of the maximal developed tension as a percentage of the maximal response to KCl, analogues **2**, **4** and **9a** displayed levels of efficacy comparable to NRB. From the data in Figure 2, it would appear that the group at C-5 plays little or no direct role in the vasoconstriction of NRB (C-5/C-7/imide), as illustrated by compound **2**, designed to mimic NRB with respect to the orientation of the group at C-7 and the *endo*-imide subunit.

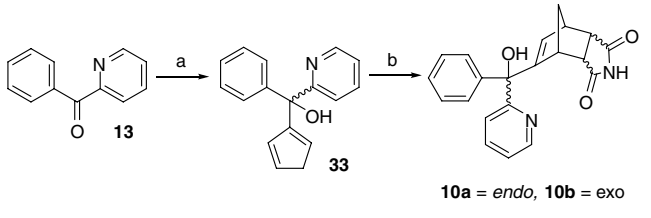
Although clearly less potent than NRB, at maximum doses analogue **2** retains similar levels of contractile activity compared to NRB. Deconstructing **2** further revealed C-7 analogue **4**, again a low potency, high efficacy vasoconstrictor. Although at this stage it would appear that the 2-pyridylbenzylidene subunit was responsible for NRB's vasoconstrictive action, further studies confirmed compounds **2** and **4** to contract in a non-NRB-type manner, concurrently confirming the importance of the group at C-5 to NRB-type contractile activity and in doing so dismissing **2** and **4** as trustworthy biological markers for lethality within this study.



Scheme 3. Reagents and conditions: (a) MeOH, cat. H_2SO_4, reflux, 18 h, 95%; (b) Br_2, CH_2Cl_2, rt, 1 h, 94%; (c) Et_3N, Et_2O, rt, 18 h, 97%; (d) DIBAL, CH_2Cl_2, 0 °C, 2 h, 51%; (e) H_2SO_4 (7.5% aq), $Na_2Cr_2O_7$, H_2O, reflux-steam distillation, 10%; (f) Br_2, CH_2Cl_2, rt, 18 h, 85%; (g) as described for step e, 40%; (h) ^{t}BuLi, Et_2O, −78 °C, 30 min then H_2O, 55%; (i) i—^{n}BuLi, Et_2O, −78 °C then, ii—**13**, Et_2O, −78 °C for 30 min warming to rt, 18 h, 28%; (j) maleimide, PhH, rt, 18 h, 47%.



Scheme 4. Reagents and conditions: (a) i—cyclopentadiene, MeMgBr, PhH, reflux, 6 h, ii—then **30**, Et_2O, 0 °C, 30 min, 33%; (b) i—**31**, NaOEt, EtOH, 0 °C, ii—then **13**, Et_2O, 0 °C, 30 min, 41%; (c) maleimide, PhH, rt, 18 h, 40%.



Scheme 5. Reagents and conditions: (a) i—cyclopentadiene, MeMgBr, PhH, reflux, 6 h, ii—then **13**, Et_2O, 0 °C, 30 min, 12%; (b) maleimide, PhH, rt, 18 h, 93%.

Indeed **2** and **4** were found to exhibit alpha adrenergic activity, since their vasoconstrictor effect was antagonized by nM concentrations of prazosin, a selective alpha-1 adrenergic receptor antagonist. Interestingly, compound **4** was found to be species-selective (active in the rat but not in the guinea pig), whereas compound **2** was not (data not shown).

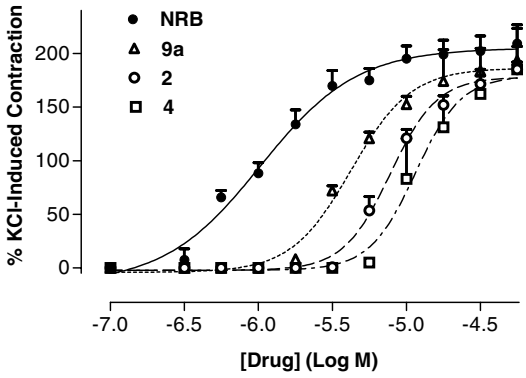


Figure 2. Contractile activity of **NRB**, **2**, **4**, and **9a** in rat caudal artery. All other compounds (**5**–**8**, **9b**, **10**–**12**) assayed were found to be inactive as vasoconstrictors. Cumulative concentration-response curves (0.1–50 μM) were constructed by expressing the contractile responses as a percentage of the contraction induced by 90 mM KCl. Each point represents the mean ± SE of at least 4 rings.

Compound **5**, closely resembling NRB with respect to the orientation of the groups at C-5 and C-7, that are conformationally locked into a rigid norbormide-type scaffold, clearly highlighted the critical role of the imide group in NRB-type vasoconstriction, being devoid of all contractile activity. Compound **5** was also found to be inactive as an alpha adrenergic agent despite incorporating the same 2-pyridylbenzylidene subunit at C-7 as present in analogues **2** and **4**, potentially the result of the group at C-5 not being tolerated in this instance. Compound **10a**, a C-5/imide analogue prepared to probe the importance of the group at C-7, was also found to be inactive as a vasoconstrictor. Indeed, of all the analogues evaluated, only **9a** was found to elicit NRB-type constriction of rat caudal artery, a result not only verifying that the 2-pyridylbenzylidene subunit at C-7, though beneficial, was non-essential; but more importantly confirming that, within this series of analogues, groups at all three sites (C-7, C-5 and imide) must be retained for NRB-type contractile activity.

In terms of a relaxing effect, analogues **5**, **6**, **9a** and **9b** were found to be vasodilators, as demonstrated by their capacity to relax rat aorta precontracted with KCl (Fig. 3). Indeed, compounds **5** and **6** were found to be equipotent to NRB.

With analogue **7** being devoid of these effects, compound **6** would appear to represent our most simplified analogue identified to date that retains such vasodilatory activity, indicating that the norbornene-backbone supporting the group at C-5 is the source of the parent compound's vasodilation action. Conversely, analogue **9a**, at the same concentration that induced the maximal contraction in the rat caudal artery, relaxes KCl contraction by only 65% of that of NRB.

Table 2. Vasoconstriction, vasodilation, mitochondrial permeability transition (stimulation of PTP opening), cardiotoxicity (coronaroconstriction) and in vivo lethality of **NRB** and compounds **2–12**

Compound	EC_{50} (μM)[a]	Maximum contractile effect[b]	Maximum relaxation effect[c]	Stimulation of PTP opening[d]	Coronaroconstriction[e]	In vivo lethality
NRB	1	209	64	++++	Yes	Yes [f]
2	7.8	193	—	++++	No	No
4	11	185	—	++++	No	No
5	—	—	60	+++	No	No
6	—	—	68	+	No	No
7	—	—	—	+	No	NT
8	—	—	—	+	No	NT
9a	4.2	185	42	++	Yes	Yes[g,h]
9b	—	—	31	++	No	NT
10a	—	—	—	+	No	NT
10b	—	—	—	+	No	NT
11	—	—	—	—	No	NT
12	—	—	—	—	No	NT

[a] EC_{50} for the contractile effect.
[b] As a % of KCl contraction (rat caudal artery).
[c] As a % of KCl contraction (rat aorta).
[d] Stimulation of the MPT as measured by the Ca^{2+} retention capacity of rat liver mitochondria in the presence of 50 μM NRB and NRB analogues (see Ref. 11), (++++ high, +++ moderate, ++ low, + negligible effect).
[e] As evidenced in Langendorff preparations in the presence of 1 μM NRB and NRB analogues.
[f] 20 mg/kg ip in rats—induced death after 1 h.
[g] 40 mg/kg ip in rats—induced death after 1 h.
[h] 40 mg/kg ip in mice—no apparent effect. NT, not tested.

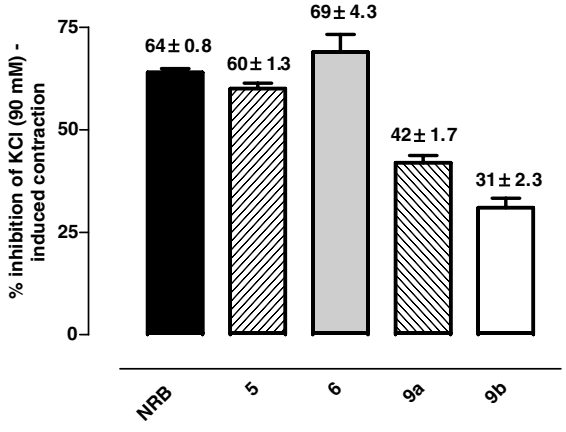


Figure 3. Vasodilation activity of **NRB**, **5**, **6**, **9a** and **9b** (at 50 μM) in rat aorta. All other compounds (**2**, **4**, **7**, **8**, and **10–12**) assayed were found to be inactive as vasodilators. Data expressed as percentage of 90 mM KCl-induced contraction. Each bar is the mean ± SE (to be calculated and added to the figure) of at least 4 rings.

With regard to mitochondrial dysfunction, compounds **2** and **4** were found to activate the permeability transition (MPT) in liver mitochondria, at levels equipotent to NRB (Table 2). Further studies confirmed compound **4** to be species-specific having no effect on either mice or guinea pig mitochondria. Compound **5** was deemed moderately active, while **9a** and **9b** were found to have only a small effect on MPT activation. All other analogues evaluated displayed negligible levels of activity. Again it is the 2-pyridylbenzylidene subunit at C-7 which appears to be the key structural feature of NRB responsible for the MPT-activating effects. In terms of cardiotoxicity, only compound **9a** was found to parallel NRB's coronaroconstriction activity (Table 2). All other analogues were found to be inactive.

Compounds **2**, **4**, **5**, **6** and **9a**, all of which elicited a positive result in vitro indicative of NRB-type activity, were subsequently evaluated in vivo to establish whether they induced death in rats (Table 2). Of these only **9a** (40 mg/kg ip) was found to induce death in rats, albeit at higher dose than NRB (20 mg/kg ip). In terms of using these in vitro indicators as biological markers for lethality in vivo, neither vasodilation nor mitochondrial dysfunction appears to be directly linked to NRB-induced death in rats, as supported by the in vivo data for compound **6** and compounds **2** and **4**, respectively.

In the absence of any clear correlation between either the in vitro contractile activity on rat peripheral artery, or coronaroconstriction, with in vivo lethality it is not possible to make any definitive conclusion about the relative roles of these factors in NRB-induced death, only to speculate that these physiological pathways may indeed be linked. Studies are currently under way to further characterize the mode of action of NRB through the design of either a pure constrictor of rat peripheral artery or a pure coronaroconstrictant.

4. Experimental

4.1. General methods (Chemistry)

All reagents were used as supplied. Solvents were purified by standard methods.[38] Analytical thin layer chromatography (TLC) was carried out on 0.20 mm pre-coated silica gel plates (ALUGRAM® SIL G/UV_{254}) and products were visualized by UV fluorescence. Flash chromatography was performed using Scharlau 60 (40–60 μm mesh) silica gel. Melting points in degres Celsius (°C) were measured on an Electrothermal®

melting point apparatus and are uncorrected. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AVANCE DRX400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) or a Bruker AVANCE 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer at 298 K. For ^{1}H NMR data, chemical shifts are described in parts per million (ppm) relative to tetramethylsilane (δ 0.00) and are reported consecutively as position (δ_H), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, qd = quartet of doublets, m = multiplet), coupling constant (*J*/Hz) and assignment. For ^{13}C NMR data, chemical shifts (ppm) are referenced internally to $CDCl_3$ (δ 77.0) and are reported consecutively as position (δ_C) and degree of hybridization. Assignments were aided by DEPT135, HSQC and HMBC experiments. When two sets of peaks arise in the NMR spectra due to the presence of isomers, the chemical shift for the minor isomer is asterisked (*). Mass spectra were recorded on a VG-70SE mass spectrometer (EI, CI and FAB). High-resolution mass spectra were recorded at a nominal resolution of 5000.

4.1.1. *cis*,*trans-endo*-7-(α-2′-Pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (2).[34] To a solution of sodium ethoxide (7.49 g, 110 mmol) in absolute ethanol (100 mL) at room temperature was added freshly distilled cyclopentadiene (7.23 g, 110 mmol), followed by the dropwise addition of 2-benzoylpyridine (**13**) (4.00 g, 21.9 mmol) in absolute ethanol (150 mL) over 2 h. The mixture was stirred for a further 30 min. and the solvent removed in vacuo. The crude residue was redissolved in dichloromethane, washed with water, the organic phase dried over anhydrous magnesium sulfate and the solvent removed in vacuo to afford 6-phenyl-6-(2′-pyridyl)fulvene (**14**)[14] as a brown oil (10 g) which was used without further purification. A solution of crude **14** (4.8 g) and maleimide (2.0 g, 20.6 mmol) in benzene (50 mL) was heated at 80 °C overnight. The resulting white solid was collected by filtration and washed with ice-cold benzene. Purification by column chromatography (hexane/ethyl acetate 1:1) afforded *endo* isomer **2** as a mixture of *cis*/*trans* isomers (1:1) (white solid; 1.67 g, 5.1 mmol, 25% over two steps). Mp 224–226 °C [lit.[34] mp 223–225 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.47 (1H, dd, *J* = 7.7 and 4.8 Hz), 3.67 (1H, dd, *J* = 7.7 and 4.8 Hz), 3.86 (1H, m), 4.39 (1H, m), 6.46–6.48 (2H, m, =CH), 6.96 (1H, d, *J* = 7.7 Hz, H-3′), 7.14 (2H, dd, *J* = 7.7 and 1.3 Hz, Ph), 7.20 (1H, qd, *J* = 7.7, 4.9 and 1.0 Hz, H-5′), 7.32–7.41 (3H, m, Ph), 7.59 (1H, dt, *J* = 7.7 and 1.3 Hz, H-4′), 7.78 (1H, s, NH), 8.65 (1H, dd, *J* = 4.9 and 1.3 Hz, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 45.6 (CH), 45.7 (CH), 46.0 (CH), 122.0 (CH), 122.5 (C), 124.5 (CH), 127.3 (CH), 128.2 (CH), 129.1 (CH), 134.0 (CH), 135.2 (CH), 136.4 (CH), 138.4 (C), 148.7 (CH), 154.8 (C), 158.2 (C), 178.1 (C), 178.3 (C); IR ($CHCl_3$) 1709 (s); *m*/*z* (EI+) 328 (M^+, 25%), 231 (100); (Found: M^+ 328.1211, $C_{21}H_{16}N_2O_2$ requires 328.1212).

4.1.2. 2′-(1-Phenylvinyl)pyridine (4)[16]. Following the procedure of Chang *et al.* a solution of **7** (2.65 g, 13.3 mmol) in aqueous sulfuric acid (10 mL, 85% v/v) was heated at 100 °C for 15 min. The reaction mixture was allowed to cool to room temperature, poured onto ice and basified through the careful addition of aqueous sodium hydroxide (50% w/v). The mixture was extracted with ethyl acetate and the combined organic phases washed with brine, then water, dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by column chromatography (hexane/ethyl acetate 3:1) afforded **4** as a thick colourless oil (2.0 g, 11.0 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 (1H, d, *J* = 1.4 Hz, =CH), 5.99 (1H, d, *J* = 1.4 Hz, =CH), 7.19 (1H, qd, *J* = 7.7, 4.5 and 1.0 Hz, H-5′), 7.27 (1H, d, *J* = 7.7 Hz, H-3′), 7.32–7.37 (5H, m, Ph), 7.63 (1H, dt, *J* = 7.7 and 1.6 Hz, H-4′), 8.64 (1H, ddd, *J* = 4.5, 1.6 and 0.7 Hz, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 117.7 (CH_2), 122.4 (CH), 122.8 (CH), 127.8 (CH), 128.3 (CH), 128.4 (CH); 136.3 (CH), 140.3 (C), 149.2 (C), 149.4 (CH), 158.5 (C); *m*/*z* (CI+) 182 (MH^+, 100%); (Found: MH^+ 182.0972, $C_{13}H_{12}N$ requires 182.0970).

4.1.3. *cis*, *trans-erythro*, *threo*-5-(α-Hydroxy-α-2-pyridylbenzyl)-7-(α-2- pyridylbenzylidene)-5-norbornene (5). A solution of **19** (8 mg, 0.02 mmol) and palladium-on-carbon (5 mg, 10 wt%) in ethyl acetate (1 mL) was stirred at room temperature under a positive atmosphere of hydrogen for 2 h. The mixture was filtered through a cotton wool plug and the solvent removed in vacuo. Purification by flash chromatography (chloroform/methanol 100:1) afforded **5** as a mixture of *cis-threo*/*trans-threo*/*cis-erythro*/*trans-erythro* isomers (1:1:1:1) (thick colourless oil; 8 mg, 0.02 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.23–1.95 (4H, m), 3.21 (0.25H, m), 3.41–3.45 (1H, m), 3.60 (0.25H, s), 3.67 (0.25H, m), 3.69 (0.25H, m), 5.54 (0.5H, dd, *J* = 3.3 and 1.0 Hz, H-6), 5.74 (0.25H, s, OH), 5.76 (0.25H, s, OH), 6.00 (0.25H, dd, *J* = 3.3 and 1.1 Hz, H-6), 6.02 (0.25H, dd, *J* = 3.3 and 1.0 Hz, H-6), 6.16 (0.25H, s, OH), 6.26 (0.25H, s, OH), 7.04–7.60 (16H, m), 8.48–8.61 (2H, m, α-Pyr); *m*/*z* (EI+) 442 (M^+, 70%), 230 (100); (Found: M^+ 442.2054, $C_{31}H_{26}N_2O$ requires 442.2045).

4.1.4. *erythro*, *threo*-5-(α-Hydroxy-α-2′-pyridylbenzyl)-5-norbornene (6). Compound **6** was prepared by a procedure similar to that of Mayo and Tam.[26] To a solution of potassium *tert*-butoxide (3.03 g, 27.0 mmol) in anhydrous tetrahydrofuran (40 mL) under nitrogen at −78 °C was added dropwise, via a cannula, norbornene (5.0 g, 53.1 mmol) in anhydrous tetrahydrofuran (12 mL), maintaining a temperature below −60 °C. *n*-Butyllithium (16.8 mL, 26.8 mmol, 1.6 M in hexanes) was added slowly over 15 min. maintaining a temperature below −70 °C. The mixture was allowed to warm to −40 °C and maintained at this temperature for 1 h. Following cooling to −70 °C a solution of anhydrous lithium bromide (3.46 g, 39.8 mmol) in anhydrous tetrahydrofuran (10 mL) was added with vigorous stirring, allowing the reaction to warm to −50 °C where it was held for a further 30 min. After cooling to −70 °C, a solution of 2-benzoylpyridine (7.29 g, 39.8 mmol) in anhydrous tetrahydrofuran (5 mL) was added. After 30 min. the reaction was quenched with water and extracted with ethyl acetate. The combined organic

phases were washed with water, dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by column chromatography (hexane/ethyl acetate 3:1) afforded **6** as a mixture of *erythro* and *threo* isomers (50:50) (white solid; 2.2 g, 7.9 mmol, 20%). Mp 84–86 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90–1.66 (6H, m), 2.79–2.84 (1.5H, m), 2.98 (0.5H, s), 5.24 (0.5H, d, J = 3.1 Hz, H-6), 5.73 (0.5H, J = 3.1 Hz, H-6), 5.82 (0.5H, s, OH), 6.18 (0.5H, s, OH), 7.04–7.40 (6H, m), 7.46 (0.5H, dt, J = 7.7 and 1.7 Hz, H-4′), 7.53–7.56 (1H, m), 7.60 (0.5H, dt, J = 7.7 and 1.7 Hz, H-4′), 8.45–8.48 (1H, m, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.1 (CH_2), 25.5 (CH_2), 26.0 (CH_2), 26.0 (CH_2), 42.3 (CH), 42.5 (CH), 43.9 (CH), 44.0 (CH), 49.0 (CH_2), 49.1 (CH_2), 78.0 (C), 78.4 (C), 121.6 (CH), 121.8 (CH), 122.0 (CH), 122.1 (CH), 126.8 (CH), 126.9 (CH), 127.0 (CH), 127.1 (CH), 127.5 (CH), 127.9 (CH), 132.1 (CH), 133.3 (CH), 135.9 (CH), 136.2 (CH), 144.4 (C), 144.8 (C), 146.8 (CH), 147.5 (CH), 152.7 (C), 153.2 (C), 161.3 (C), 162.2 (C); IR ($CHCl_3$) 3430 (br); *m/z* (EI+) 277 (M^+, 100%); (Found: M^+ 277.1472, $C_{19}H_{19}NO$ requires 277.1467).

4.1.5. 1-Phenyl-1-(pyridine-2′-yl)ethanol (7).[17] To a solution of 2-benzoylpyridine (2.5 g, 13.7 mmol) in anhydrous tetrahydrofuran (30 mL) under nitrogen at room temperature was added dropwise methylmagnesium bromide (5.0 mL, 15.1 mmol, 3.0 M in diethyl ether), and the mixture stirred for 18 h. The reaction was quenched by pouring onto a mixture of ice and aqueous hydrochloric acid (1 M), basified through the addition of solid potassium carbonate and extracted with ethyl acetate. The combined organic phases were washed with brine, then water, dried over anhydrous magnesium sulfate and the solvents removed in vacuo. Purification by column chromatography (hexane/ethyl acetate 3:1) afforded **7** as a thick colourless oil (2.59 g, 13.0 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (3H, s, CH_3), 7.19–7.37 (5H, m), 7.51–7.54 (2H, m), 7.67 (1H, dt, J = 7.8 and 1.6 Hz, H-4′), 8.56 (1H, m, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.2 (CH_3), 75.1 (C), 120.3 (CH), 122.0 (CH), 125.8 (CH), 126.9 (CH), 128.2 (CH), 137.0 (CH), 147.1 (C), 147.3 (CH), 164.7 (C); IR ($CHCl_3$) 3429 (br); *m/z* (EI+) 199 (M^+, 100%); (Found: M^+ 199.0994, $C_{13}H_{13}NO$ requires 199.0997).

4.1.6. *endo*,*exo-threo*-7-Oxa-[5-(α-hydroxy-α-2′-pyridylbenzyl)]-5-norbornene-2,3-dicarboximide (8). A solution of **26** (200 mg, 0.8 mmol) and maleimide (66 mg, 0.68 mmol) in benzene (5 mL) was stirred at room temperature for 3 days. The solvent was removed in vacuo to afford a mixture of *endo* **8a** and *exo* **8b** isomers (2:98). Purification by column chromatography (hexane/ethyl acetate 1:1) afforded *exo* isomer **8b** exclusively as the *threo* isomer[39] (thick colourless oil; 112 mg, 0.32 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.97 (1H, d, J = 6.6 Hz), 3.09 (1H, d, J = 6.6 Hz), 5.10 (1H, s), 5.30 (1H, d, J = 1.8 Hz), 5.87 (1H, s, OH), 6.15 (1H, d, J = 1.8 Hz, H-6), 7.28–7.39 (6H, m), 7.47 (1H, m), 7.80 (1H, dt, J = 7.7 and 1.7 Hz, H-4′), 8.60 (1H, m, H-6′), 8.76 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 49.3 (CH), 49.9 (CH), 76.9 (C), 82.1 (CH), 82.4 (CH), 121.4 (CH), 123.2 (CH), 126.5 (CH), 127.7 (CH), 128.2 (CH), 134.2 (CH), 137.4 (CH), 143.0 (C), 148.4 (CH), 155.5 (C), 160.3 (C), 176.6 (C), 176.7 (C); IR ($CHCl_3$) 1713 (s); *m/z* (EI+) 348 (M^+, 8%), 251 (100); (Found: M^+ 348.1100, $C_{20}H_{16}N_2O_4$ requires 348.1110).

4.1.7. *endo*, *exo-erythro*, *threo*-5-(α-Hydroxy-α-2′-pyridylbenzyl)-7-(α-phenylbenzylidene)-5-norbornene-2,3-dicarboximide (9). A solution of **32** (0.83 g, 2.0 mmol) and maleimide (0.19 g, 2.0 mmol) in benzene (5 mL) was heated at reflux overnight. The solvent was removed in vacuo to afford a mixture of *endo* **9a** and *exo* **9b** isomers (1.2:1). Purification by column chromatography (hexane/ethyl acetate 1:1) afforded *endo* isomer **9a** as a mixture of *erythro**/*threo* isomers[39] (15:85) (white solid; 225 mg, 0.44 mmol, 22%); and *exo* isomer **9b** as the *erythro* isomer[39] (>95%) (white solid; 184 mg, 0.36 mmol, 18%). **9a**: mp 210–212 °C [lit.[40] mp (mixed isomers) 205–207 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.44* (0.15H, dd, J = 7.9 and 4.5 Hz), 3.49 (0.85H, dd, J = 7.8 and 4.5 Hz), 3.53–3.56 (1H, m), 3.74* (0.15H, m), 3.89 (0.85H, m), 3.93* (0.15H, m), 4.08 (0.85H, m), 5.64 (0.85H, dd, J = 3.3 and 1.2 Hz, H-6), 5.72* (0.15H, s, OH), 5.83 (0.85H, s, OH), 6.11* (0.15H, dd, J = 3.3 and 1.2 Hz, H-6), 6.86–7.54 (18H, m), 8.25 (1H, s, NH), 8.48–8.50 (1H, m, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 45.6* (CH), 45.8 (CH), 46.1* (CH), 46.5 (CH), 48.0 (CH), 48.4* (CH), 49.1 (CH), 49.3* (CH), 77.2* (C), 77.7 (C), 121.9 (CH), 122.5 (CH), 122.6* (CH), 123.2* (C), 127.0* (CH), 127.1 (CH), 127.2 (CH), 127.4 (CH), 127.6 (CH), 127.9* (CH), 128.0 (CH), 128.1 (CH), 128.2 (CH), 129.1* (CH), 129.2 (CH), 129.3 (CH), 129.8 (CH), 133.0* (CH), 136.4 (CH), 136.5* (CH), 139.6 (C), 139.9 (C), 142.5 (C), 147.9 (CH), 148.2* (CH), 151.7 (C), 152.3* (C), 155.1 (C), 160.7 (C), 176.4* (C), 176.6 (C), 176.9 (C); IR ($CHCl_3$) 1710 (s); *m/z* (FAB+) 511 (MH^+, 8%), 154 (100); (Found: MH^+ 511.2013, $C_{34}H_{27}N_2O_3$ requires 511.2022). **9b**: mp 102–104 °C [lit.[40] mp (mixed isomers) 205–207 °C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.93 (1H, dd, J = 7.4 and 0.7 Hz), 2.99 (1H, dd, J = 7.4 and 0.7 Hz), 3.59 (1H, s), 3.82 (1H, m), 5.84 (1H, s, OH), 6.21 (1H, dd, J = 3.4 and 0.9 Hz, H-6), 6.89–6.94 (2H, m), 7.04–7.07 (2H, m), 7.13–7.30 (13H, m), 7.49 (1H, dt, J = 7.7 and 1.7 Hz, H-4′), 8.32 (1H, s, NH), 8.50 (1H, m, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.6 (CH), 49.1 (CH), 50.7 (CH), 78.6 (C), 121.8 (CH), 122.7 (CH), 127.0 (CH), 127.1 (CH), 127.2 (CH), 127.5 (CH), 128.0 (CH), 128.2 (CH), 128.8 (CH), 129.0 (CH), 134.7 (CH), 136.8 (CH), 139.7 (C), 139.8 (C), 142.7 (C), 144.9 (C), 148.2 (CH), 156.7 (C), 160.6 (C), 176.9 (C), 177.4 (C); IR ($CHCl_3$) 1708 (s); *m/z* (FAB+) 511 (MH^+, 7%), 154 (100); (Found: MH^+ 511.2015, $C_{34}H_{27}N_2O_3$ requires 511.2022).

4.1.8. *endo*, *exo-erythro*, *threo*-5-(α-Hydroxy-α-2′-pyridylbenzyl)-5-norbornene-2,3-dicarboximide (10). A solution of **33** (156 mg, 0.63 mmol) and maleimide (61 mg, 0.63 mmol) in benzene (3 mL) was stirred at room temperature overnight. The solvent was removed in vacuo to afford a mixture of *endo* **10a** and *exo* **10b** isomers (6:1). Purification by column chromatography (hexane/ethyl acetate 2:1) afforded *endo* isomer **10a** as a mixture of *erythro**/*threo* isomers[39] (1:2) (white solid; 173 mg,

0.5 mmol, 79%); and *exo* isomer **10b** as a mixture of *erythro*/*threo**isomers[39] (55:45) (white solid; 30 mg, 0.09 mmol, 14%). **10a**: mp 196–198 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.64* (0.33H, d, J = 8.7 Hz), 1.66 (0.66H, d, J = 8.7 Hz), 2.06* (0.33H, d, J = 8.7 Hz), 2.10 (0.66H, d, J = 8.7 Hz), 3.26–3.46 (3H, m), 3.58–3.60 (1H, m), 5.45 (0.66H, d, J = 3.1 Hz, H-6), 5.64* (0.33H, s, OH), 5.84* (0.33H, d, J = 3.1 Hz, H-6), 5.91 (0.66H, s, OH), 7.01 (0.66H, d, J = 7.5 Hz), 7.15 (0.66H, qd, J = 7.5, 5.0 and 1.1 Hz, H-5′), 7.22–7.40 (4H, m), 7.51* (0.33H, d, J = 7.9 Hz), 7.55 (0.66H, dt, J = 7.7 and 1.7 Hz, H-4′), 7.60 (1.33H, dd, J = 8.8 and 1.6 Hz), 7.76* (0.33H, dt, J = 7.7 and 1.7 Hz, H-4′), 8.10 (0.66H, s, NH), 8.38* (0.33H, s, NH), 8.50 (0.66H, m, H-6′), 8.58* (0.33H, m, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 44.3* (CH), 44.4 (CH), 47.7* (CH), 47.8 (CH), 48.8 (CH), 48.9* (CH), 53.8 (CH_2), 54.3* (CH_2), 77.3 (C), 121.5* (CH), 121.7 (CH), 122.3 (CH), 122.6* (CH), 126.7* (CH), 127.1 (CH), 127.5 (CH), 127.8* (CH), 128.1 (CH), 130.3 (CH), 133.6* (CH), 136.3 (CH), 136.7* (CH), 143.2 (C), 143.7* (C), 147.7 (CH), 148.2* (CH), 153.8* (C), 154.4 (C), 161.1 (C), 161.4* (C), 177.6 (C), 177.7 (C); IR ($CHCl_3$) 1709 (s); *m*/*z* (EI+) 346 (M^+, 100%); (Found: M^+ 346.1321, $C_{21}H_{18}N_2O_3$ requires 346.1317). **10b**: mp 208–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49–1.54 (1H, m), 1.76–1.85 (1H, m), 2.76* (0.45H, dd, J = 7.1 and 0.9 Hz), 2.80 (0.55H, d, J = 7.1 Hz), 2.91 (0.55H, m), 3.13–3.15 (1H, m), 3.28–3.32 (1H, m), 3.38* (0.45H, m), 5.50* (0.45H, d, J = 3.1 Hz, H-6), 5.94 (0.55H, s, OH), 5.95 (0.55H, d, J = 3.1 Hz, H-6), 6.24* (0.45H, s, OH), 7.10* (0.45H, dd, J = 8.0 and 1.0 Hz, H-3′), 7.21–7.38 (5.65H, m), 7.45–7.48 (0.9H, m), 7.61* (0.45H, dt, J = 7.6 and 1.8 Hz, H-4′), 7.75 (0.55H, dt, J = 7.7 and 1.7 Hz, H-4′), 8.17 (1H, s, NH), 8.53–8.57 (1H, m, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 43.2 (CH), 45.8 (CH), 46.0* (CH), 47.0 (CH), 47.2* (CH), 49.3 (CH), 49.5* (CH), 50.2 (CH_2), 77.9* (C), 78.2 (C), 121.4 (CH), 122.1* (CH), 122.5* (CH), 122.8 (CH), 126.7 (CH), 126.8 (CH), 127.4* (CH), 127.6* (CH), 128.0 (CH), 128.4* (CH), 133.1* (CH), 135.4 (CH), 136.7* (CH), 137.0 (CH), 143.2 (C), 143.7* (C), 147.3* (CH), 148.2 (CH), 155.5 (C), 156.0* (C), 160.4* (C), 161.1 (C), 177.9 (C), 178.0* (C), 178.1 (C); IR ($CHCl_3$) 1708 (s); *m*/*z* (EI+) 346 (M^+, 100%); (Found: M^+ 346.1319, $C_{21}H_{18}N_2O_3$ requires 346.1317).

4.1.9. *cis*, *trans-erythro*, *threo*-5-(α-Hydroxy-α-2-pyridylbenzyl)-7-(α-2- pyridylbenzylidene)-2,5-norbornadiene (19). A solution of **15** (2.07 g, 5.0 mmol) and (*E*)-1-benzenesulfonyl-2-(trimethylsilyl)ethylene (**21**) (1.21 g, 5.0 mmol) in toluene (20 mL) was heated at reflux for 2 days. The solvent was removed in vacuo with partial purification by column chromatography (hexane/ethyl acetate 5:1) affording an isomeric mixture of **20** as a pale brown solid (0.71 g, ca. 1.1 mmol, ca. 22%). A solution of **20** (0.52 g, ca. 0.8 mmol) and *tetra*-butylammonium fluoride hydrate (1.05 g, 4.0 mmol) in tetrahydrofuran (4 mL) was heated at reflux for 2 days. The solvent was removed in vacuo, with purification by column chromatography (hexane/ethyl acetate 5:1) affording **19** as a mixture of *cis-threo*/*trans-threo*/*cis-erythro*/*trans-erythro* isomers (1:1:1:1) (thick colourless oil; 70 mg, 0.16 mmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.95 (0.25H, m), 4.00 (0.25H, m), 4.06–4.08 (0.5H, m), 4.24 (0.25H, m), 4.26 (0.25H, m), 4.47 (0.25H, m), 4.54 (0.25H, m), 5.97 (0.25H, s, OH), 6.00 (0.25H, s, OH), 6.12 (0.25H, dd, J = 3.4 and 0.9 Hz, H-6), 6.14 (0.25H, dd, J = 3.5 and 1.1 Hz, H-6), 6.33 (0.25H, dd, J = 3.5 and 1.1 Hz, H-6), 6.36 (0.25H, dd, J = 3.4 and 1.1 Hz, H-6), 6.40 (0.25H, s, OH), 6.61 (0.25H, s, OH), 6.91–7.52 (18H, m), 8.51–8.54 (2H, m, α-Pyr); *m*/*z* (FAB+) 441 (MH^+, 9%), 154 (100); (Found: MH^+ 441.19592, $C_{31}H_{25}N_2O$ requires 441.1967).

4.1.10. *R*,*S*-3-(α-Hydroxy-α-2′-pyridylbenzyl)furan (26). To a stirred solution of 3-bromofuran (0.5 mL, 5.56 mmol) in anhydrous tetrahydrofuran (8 mL) under nitrogen at −78 °C was added dropwise *n*-butyllithium (3.5 mL, 5.56 mmol, 1.6 M in hexanes) and the temperature maintained for 30 min. 2-Benzoylpyridine (1.12 g, 6.12 mmol) in anhydrous tetrahydrofuran (5 mL) was added slowly and the reaction was allowed to warm to room temperature overnight. The reaction was quenched with a saturated aqueous solution of ammonium chloride (1 mL), diluted with water and extracted with diethyl ether. The combined organic phases were washed with brine, then water, dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by column chromatography (hexane/ethyl acetate 5:1) afforded **26** as a colourless oil (0.39 g, 1.55 mmol, 28%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.24 (1H, s), 6.34 (1H, t, J = 0.7 Hz), 7.03 (1H, t, J = 0.7 Hz), 7.11 (1H, dd, J = 7.6 and 5.0 Hz, H-5′), 7.22–7.29 (4H, m), 7.34 (1H, t, J = 1.6 Hz), 7.40–7.43 (2H, m), 7.54 (1H, dt, J = 7.6 and 1.6 Hz, H-4′), 8.47 (1H, d, J = 5.0 Hz, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 75.6 (C), 110.2 (CH), 121.4 (CH), 122.1 (CH), 126.8 (CH), 127.0 (CH), 127.6 (CH), 131.4 (C) 136.3 (CH), 140.7 (CH), 142.9 (CH), 145.0 (C), 147.1 (CH), 162.2 (C); *m*/*z* (EI+) 251 (M^+, 100%); (Found: M^+ 251.0943, $C_{16}H_{13}NO_2$ requires 251.0946).

4.1.11. 2-(α-Hydroxy-α-2-phenylbenzyl)cyclopenta-1,3-diene (31a) and 1-(α-hydroxy-α-2-phenylbenzyl)cyclopenta-1,3-diene (31b)[35]. To a stirred solution of methylmagnesium bromide (6.6 mL, 20.0 mmol, 3.0 M in diethyl ether) under nitrogen was added benzene (12.5 mL), and the diethyl ether distilled off until the boiling point of the mixture reached 63 °C. Freshly distilled cyclopentadiene (1.32 g, 20.0 mmol) was then added and the mixture heated at reflux for 6 h. The resultant cyclopentadienylmagnesium bromide solution was then cooled to 0 °C and benzophenone (3.66 g, 20.0 mmol) in diethyl ether (10 mL) added, while maintaining the temperature at 0 °C. After stirring at 0 °C for 30 min., the mixture was hydrolysed with an excess of ice water containing glacial acetic acid (1.2 g) and extracted with diethyl ether. The combined organic phases were washed with an aqueous solution of sodium hydrogen carbonate, dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification by column chromatography (hexane/ethyl acetate 10:1) afforded a regioisomeric mixture of **31a*** and **31b** (1:3) as a white solid (1.63 g, 6.57 mmol, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.85 (1H, s, OH), 2.98 (2H, s, H-5), 5.91* (0.25H, d,

J = 1.2 Hz, H-1), 6.06 (0.75H, d, J = 1.0 Hz), 6.33–6.36 (1.5H, m), 6.40* (0.25H, m), 6.44* (0.25H, m), 7.16–7.36 (10H, m, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.9* (CH_2), 41.5 (CH_2), 79.0* (C), 79.9 (C), 125.7* (CH), 126.7* (CH), 127.0 (CH), 127.1 (CH), 127.2 (CH), 127.7 (CH) 128.0* (CH), 129.7* (CH), 131.2 (CH), 133.1* (CH), 133.4 (CH), 133.8* (CH), 145.7* (C) 146.4 (C), 152.1* (C), 153.6 (C); *m/z* (FAB+) 248 (M^+, 25%), 154 (100); (Found: M^+ 248.1192, $C_{18}H_{16}O$ requires 248.1201).

4.1.12. *Erythro*, *threo*-6,6,α-Triphenyl-α-(2′-pyridyl)-2-fulvenylmethanol (32)[35]. To a stirred solution of **31a** and **31b** (1.63 g, 6.57 mmol) and 2-benzoylpyridine (1.2 g, 6.57 mmol) in absolute ethanol (100 mL) under nitrogen at 0 °C was added a solution of sodium ethoxide (0.45 g, 6.57 mmol) in absolute ethanol (13 mL), and the mixture stirred at 0 °C for 3 h. The resulting orange solid was collected by filtration, washed with ethanol and dried in vacuo to afford **32** (1.1 g, 2.66 mmol, 41%) which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.94 (1H, t, J = 2.0 Hz, H-1), 6.17 (1H, s, OH), 6.32 (1H, dd, J = 5.4 and 2.0 Hz, H-4), 6.57 (1H, dd, J = 5.4 and 1.6 Hz, H-3), 7.19–7.37 (15H, m), 7.45 (2H, d, J = 7.7 and 1.5 Hz), 7.62 (1H, dt, J = 7.5 and 1.6 Hz, H-4′), 8.56 (1H, m, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 78.1 (C), 121.2 (CH), 122.2 (CH), 122.3 (CH), 125.3 (CH), 127.3 (CH), 127.5 (CH), 127.6 (CH), 128.0 (CH), 128.8 (CH), 131.9 (CH), 132.9 (CH), 136.5 (CH), 141.0 (C), 141.2 (C), 143.0 (C), 144.9 (C), 147.4 (CH), 151.8 (C), 162.1 (C); *m/z* (EI+) 413 (M^+, 5%), 395 (100, M^+ - H_2O); (Found: M^+ 413.1777, $C_{30}H_{23}NO$ requires 413.1780).

4.1.13. *R,S*-2-(α-Hydroxy-α-2′-pyridylbenzyl)cyclopenta-1,3-diene (33). A similar procedure[35] to that described above for the preparation of **31** was followed using 2-benzoylpyridine (3.66 g, 20 mmol) to afford a regioisomeric mixture of 1-(α-hydroxy-α-2′-pyridylbenzyl)cyclopenta-1,3-diene and **33** (3:2). Purification by column chromatography (hexane/ethyl acetate 20:1) afforded **33**as a thick pale orange oil (0.61 g, 2.45 mmol, 12%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.00 (2H, t, J = 1.3 Hz, H-5), 5.85 (1H, t, J = 1.7 Hz, H-1), 6.20 (1H, s, OH), 6.44 (1H, m), 6.53 (1H, m), 7.14–7.31 (5H, m), 7.40–7.42 (2H, m), 7.58 (1H, dt, J = 7.8 and 1.8 Hz, H-4′), 8.51 (1H, m, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 41.0 (CH_2), 77.6 (C), 122.1 (CH), 122.2 (CH), 127.1 (CH), 127.2 (CH), 127.8 (CH), 129.8 (CH), 133.2 (CH), 133.7 (CH), 136.4 (CH), 144.8 (C), 147.4 (CH), 151.4 (C), 162.1 (C); *m/z* (EI+) 249 (M^+, 35%), 231 (100); (Found: M^+ 249.1151, $C_{17}H_{15}NO$ requires 249.1154).

4.2. General methods (Pharmacology)

4.2.1. Rat caudal artery and aortic ring isolation and recording of contractile force. Male Wistar rats (150–250 g) were obtained from Charles River Italia (Milano, Italy) and killed by decapitation. Arteries (thoracic aorta and ventral caudal artery) were collected, placed in Tyrode solution at room temperature and cleaned of extraneous fatty and connective tissue under a dissection microscope. All vessels were cut into rings 2 mm long, mounted on a custom-built plexiglass support by means of two intraluminal tungsten wires and placed in 20 mL double-jacketed organ baths filled with Tyrode solution of the following composition (mM): NaCl 125, KCl 5, $CaCl_2$ 2.7, $MgSO_4$ 1, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11, maintained at 37 °C, pH 7.35, bubbled with 95% O_2 and 5% CO_2. The endothelium was removed by gently rubbing the lumen of the rings with a round wooden stick (aorta) or a very thin rough-surfaced tungsten wire (caudal artery). The mechanical activity of the rings was detected by means of an isometric force transducer (Ugo Basile, Comerio, Italy) coupled to a pen recorder (Ugo Basile, Comerio, Italy). Rings were passively stretched to impose a resting tension (1.5–2 g) and were allowed to equilibrate for 60 min. After equilibration, each ring was repeatedly stimulated with 10 μM phenylephrine until a reproducible response was obtained. To verify the absence of the endothelium, rings contracted with 1 μM phenylephrine were exposed to 2 μM carbamylcholine. The absence of the endothelium was revealed by the lack of carbamylcholine-induced relaxation. Concentration-response curves (0.1–50 μM) were produced for each compound to assess the contractile effect on caudal artery. The inibitory effect was evaluated in the aorta by measuring the contractile response to 90 mM KCl in the absence and presence of maximal concentrations of the compound, and was expressed by the following formula: $100 \times (C - AC)/C$, where C and AC are the contractile effects measured in milligrams of KCl alone and in the presence of the antagonist, respectively. In a second set of experiments dose-response curves were made for all the compounds in presence of several antagonists, to assess norbormide-like behaviour. In particular, prazosin, a selective alpha-1 adrenergic receptor antagonist, was used at concentrations ranging from 1 to 10 nM.

4.2.2. Mitochondrial permeability transition (MPT). Mitochondria from the liver of Albino Wistar rats were prepared according to standard differential centrifugation procedures.[10] The final pellet was suspended in Tris/HCl buffer, pH 7.4, containing 0.25 M sucrose and 0.1 mM EGTA to give a protein concentration of 80–100 mg/mL, as measured using the biuret method. The functionality of mitochondria was established by measuring the value of the respiratory control ratio (RCR), the ratio between the rate of oxygen consumption in state 3 (in the presence of 0.3 mM ADP) and in state 4 (in the absence of ADP) in a thermostated (T = 25 °C), waterjacketed vessel, using a Clark electrode connected to a recorder. The incubation buffer contained 10 mM Tris–MOPS, 100 mM sucrose, 2 mM $MgCl_2$, 50 mM KCl, 10 mM KH_2PO_4, 1 mM EDTA and 2 μM rotenone, pH 7.4. Succinate (5 mM) was used as the energizing substrate. Only mitochondrial suspensions with a RCR $\geqslant$ 3.0 were considered. MPT was generally induced at 25 °C in a medium containing 200 mM sucrose, 10 mM Tris–MOPS, 10 μM EGTA–Tris, 1 mM P_i, 0.5 μg/mL oligomycin, 5 mM succinate and 2 μM rotenone, pH 7.4 (standard medium).[41] Ca^{2+} was used as MPT inducer. The MPT-stimulating effects of NRB

analogues were investigated by following the Ca^{2+} retention capacity (CRC) of mitochondria, i.e., the amount of calcium that can be accumulated and retained by mitochondria until the MPT occurs. The CRC of mitochondrial preparations was assessed fluorimetrically in the presence of the Ca^{2+} indicator Calcium Green-5N (Molecular Probes) with a Perkin Elmer LS50B spectrofluorimeter exactly as described previously.[10]

4.2.3. Langendorff preparation. Male Wistar rats (150–250 g) were heparinised and sacrificed by decapitation. The hearts were quickly removed, incannulated through the aorta and perfused at constant flow (10 mL/min) with a Krebs–Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11 and Na pyruvate 2, maintained at 37 °C, pH 7.35, bubbled with 95% O_2 and 5% CO_2. The heart was electrically driven at a frequency of 6 Hz through platinum electrodes placed on the left atrium. Left ventricular developed pressure (LVDP) was measured by inserting in the left ventricle a steel cannula connected to a pressure transducer (2B instruments, Italy). Coronary pressure (CO) was recorded by means of a pressure transducer (2B instruments, Italy) positioned just before the aortic cannula. Each compound was tested by infusion at a final concentration of 5 μM. Hemodynamic data were collected by a real-time digital acquisition and analysis system (model MP-100, Biopac System, Santa Barbara (CA)) and displayed on a monitor during the experiment. LVDP and CO were expressed as mmHg.

4.2.4. In vivo experiments. Four male Wistar rats (150–250 g) were used to test the lethality of each compound. Briefly, all the drugs were dissolved in 200 μM of DMSO to obtain a final concentration corresponding to 40 mg/kg and injected into the peritoneal cavity. All the treated animals were housed in a quiet room and monitored every 15 min to verify early signs of toxicity. The concentration used was arbitrarily chosen as the double of that of NRB. In parallel, four rats were injected with the same volume of solvent alone as a control.

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References and notes

1. *Handbook of Pesticide Toxicology*, Vol. 2, Agents, In 2nd ed.; Krieger, R. I., Ed.; Academic Press, 2001.
2. Przyborowski, T.; Hillar, M. Biul. *Inst. Med. Morsk Gdansk* **1968**, *19*, 211–216.
3. Rennison, B. D.; Hammond, L. E.; Jones, G. L. *J. Hygiene* **1968**, *66*, 147–158.
4. Roszkowski, A. P. *J. Pharm. Exp. Ther.* **1965**, *149*, 288–299.
5. Bova, S.; Cima, L.; Golovina, V.; Luciani, S.; Cargnelli, G. *Cardiovasc. Drug Rev.* **2001**, *19*, 226–233.
6. Brimble, M. A.; Muir, V. J.; Hopkins, B.; Bova, S. *Arkivoc* **2004**, 1–11.
7. Poos, G. I.; Mohrbacher, R. J.; Carson, E. L.; Paragamian, V.; Puma, B. M.; Rasmussen, C. R.; Roszkowski, A. P. *J. Med. Chem.* **1966**, *9*, 537–540.
8. Bova, S.; Trevisi, L.; Cima, L.; Luciani, S.; Golovina, V.; Cargnelli, G. *J. Pharm. Exp. Ther.* **2001**, *296*, 458–463.
9. Fusi, F.; Saponara, S.; Sgaragli, G.; Cargnelli, G.; Bova, S. *Br. J. Pharmacol.* **2002**, *137*, 323–328.
10. Ricchelli, F.; Dabbeni-Sala, F.; Petronilli, V.; Bernardi, P.; Hopkins, B.; Bova, S. *BBA Bioenerg.* **2005**, *1708*, 178–186.
11. Yelnosky, J.; Lawlor, R. *Eur. J. Pharmacol.* **1971**, *16*, 117–119.
12. For earlier structure–activity studies on NRB, see Ref. 7.
13. Roszkowski, A. P.; Poos, G. I.; Mohrbacher, R. J. *Science* **1964**, *144*, 412–413.
14. Mohrbacher, R. J.; Paragamian, V.; Carson, E. L.; Puma, B. M.; Rasmussen, C. R.; Meschino, J. A.; Poos, G. I. *J. Org. Chem.* **1966**, *31*, 2149–2159.
15. *endo*/*exo* Assignments were made on the basis of coupling constants ($J_{1,2endo}$ = 4.8–5.0 Hz). Davis, J. C., Jr.; Van Auken, T. V. *J. Am. Chem. Soc.* **1965**, *87*, 3900–3905, and Ref. 6.
16. Chang, C. J.; Kiesel, R. F.; Hogen-Esch, T. E. *J. Am. Chem. Soc.* **1975**, *97*, 2805–2810.
17. Gomez, I.; Alonso, E.; Ramon, D. J.; Yus, M. *Tetrahedron* **2000**, *56*, 4043–4052.
18. Adamson, D. W.; Barrett, P. A.; Billinghurst, J. W.; Jones, T. S. G. *J. Chem. Soc.* **1958**, 312–324.
19. Carr, R. V. C.; Williams, R. V.; Paquette, L. A. *J. Org. Chem.* **1983**, *48*, 4976–4986.
20. Mohrbacher, R. J.; Poos, G. I.; Roszkowski, A. P. French Patent 1386735, 1965.
21. Mohrbacker, R. J.; Poos, G. I.; Roszkowski, A. P. Belgian Patent 659610, 1965.
22. Snow, R. A.; Degenhardt, C. R.; Paquette, L. A. *Tetrahedron Lett.* **1976**, *49*, 4447–4450.
23. Sheldon, R. A.; Kochi, J. K. *Org. React.* **1972**, *19*, 279.
24. Cain, E. N.; Vukov, R.; Masamune, S. *Chem. Commun.* **1969**, *98*.
25. Pillot, J.-P.; Dunogues, J.; Calas, R. *Synthesis* **1977**, *7*, 469.
26. Mayo, P.; Tam, W. *Tetrahedron* **2002**, *58*, 9527–9540.
27. Jones, G.; Fantina, M. E.; Pachtman, A. H. *J. Org. Chem.* **1976**, *41*, 329–333.
28. Efskind, J.; Benneche, T.; Undheim, K. *Acta Chem. Scand.* **1997**, *51*, 942–952.
29. Kraus, G. A.; Wang, X. *Synth. Commun.* **1998**, *28*, 1093–1096.
30. Alvarez-Ibarra, C.; Quiroga, M. L.; Toledano, E. *Tetrahedron* **1996**, *52*, 4065–4078.
31. Hartman, G. D.; Halczenko, W.; Phillips, B. T. *J. Org. Chem.* **1986**, *51*, 142–148.
32. Akhrem, A. A. *Bull. Acad. Sci. USSR Div. Chem. Sci.* **1960**, 654–661 (Eng. Transl.).
33. Gorzynsky, M.; Rewicki, D. *Leibigs Ann. Chem.* **1986**, *4*, 625–637.
34. (a) Poos, G. I.; Lehman, M. M.; Landis, E. B.; Rosenau, J. D. *J. Med. Pharm. Chem.* **1962**, *5*, 883–896; (b) Mohrbacher, R. J.; Almond, H. R.; Carson, E. L.; Rosenau, J. D.; Poos, G. I. *J. Org. Chem.* **1966**, *31*, 2141–2148.
35. Mohrbacher, R. J.; Poos, G. I. U.S. Patent 3,376,304, 1968.
36. HMBC studies confirmed the formation of compound **32** through a strong 3-bond coupling of the carbinol carbon at C-5 with proton H-6 of the pyridyl ring.

37. Bova, S.; Trevisi, L.; Debetto, P.; Cima, L.; Furnari, M.; Luciani, S.; Padrini, R.; Cargnelli, G. *Br. J. Pharmacol.* **1996**, *117*, 1041–1046.
38. Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon Press: Oxford, 1980.
39. The pyridyl group attached to the carbinol carbon can deshield H-6 more strongly when the arrangement about the carbinol carbon is *erythro*. Hence H-6 *erythro* resonates further downfield compared to H-6 *threo*. *erythro*/*threo* designations were assigned on this basis. See Ref. 6.
40. Mohrbacher, R. J.; Poos, G. I.; Roszkowski, A. P. U.S. Patent 3,378,566, 1968.
41. Petronilli, V.; Cola, C.; Massari, S.; Colonna, R.; Bernardi, P. *J. Biol. Chem.* **1993**, *268*, 21939–21945.