Articles

Benzylguanidines and Other Galegine Analogues Inducing Weight Loss in Mice

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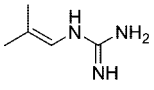
Dimethylallylguanidine, also known as galegine, isolated from *Galega officinalis*, has been shown to have weight reducing properties in vivo. Substitution of the guanidine group with an *N*-cyano group and replacement of guanidine with amidine, pyrimidine, pyridine, or the imidazole moieties removed the weight reducing properties when evaluated in BALB/c mice. However, retention of the guanidine and replacement of the dimethylallyl group by a series of functionalized benzyl substituents was shown to exhibit, and in some cases significantly improve, the weight reducing properties of these molecules in BALB/c, *ob*/*ob*, and diet induced obesity (DIO) mice models. The lead compound identified, across all models, was 1-(4-chlorobenzyl)guanidine hemisulfate, which gave an average daily weight difference (% from time-matched controls; ± SEM) of −19.7 ± 1.0, −11.0 ± 0.7, and −7.3 ± 0.8 in BALB/c, *ob*/*ob*, and DIO models, respectively.

Introduction

Dimethylallylguanidine, also known as galegine (**1**), was first isolated from the plant *Galega officinalis* in the late 1800s.[1] The plant has a long history of use as an antidiabetic,[2] and galegine itself was used for a short time as a hypoglycemic.[1] In the 1950s, the "formin" class of agents, used for the treatment of type 2 diabetes,[3,4] were discovered, although these are biguanides rather than guanidines, which changes their properties very substantially, both biologically and chemically.[5] The discovery of hypoglycemic activity in the biguanides appears to have been by chance rather than through deliberate structural modification of the guanidine lead.[6] Metformin (**2**) is still used to treat diabetes, the main problems being the large doses required and the high incidence of gastrointestinal disturbance. Phenformin (**3**) was initially a more popular drug than metformin but was controversially abandoned[7] in the 1970s owing to lactic acidosis, a rare but serious side effect.[8]



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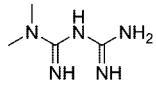

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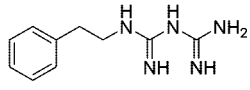

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Galegine was reisolated following an investigation of *Galega officinalis* as part of an antidiabetic herbal mixture[9] and the observation that mice fed with the plant mixture did not gain weight, despite eating normally and showing no signs of toxicity. Bioassay guided fractionation led to the identification of galegine as the main active ingredient. Prior to this, a varied selection of structures, including guanidines, had been screened for antidiabetic and weight-loss activity;[10] it is difficult to draw structure−action relationships from these published data, however, owing to the heterogeneity of the structures tested. More recently, pyrazinoylguanidine[11] has been described as a potential treatment for diabetics who also suffer from hypertension. Importantly, we have recently reported that the effects of galegine, including the enhanced uptake of glucose and the inhibition of acetyl-CoA carboxylase, can be explained by the activation of AMP activated protein kinase (AMPK[a]).[12] As the inhibition of acetyl-CoA carboxylase both inhibits fatty acid synthesis and stimulates fatty acid oxidation,[13] we were interested in exploring whether galegine analogues might form the basis of drugs for the potential treatment of obesity.

Synthesis. Guanidines can be readily prepared from amines by reaction with 2-methyl-2-thiopseudourea sulfate (Scheme 1).[14] Many of the amines used in the present study were commercially available; others were synthesized by short routes from readily available precursors. Galegine was synthesized for this study rather than being isolated from the plant. Guanidines are very strong bases, which can lead to problems in isolation because they may not be extractable from aqueous solution even when the solution is made strongly alkaline. For the same reason, they tend to react with atmospheric carbon dioxide, forming a molecule of a carbamic acid, which then reacts with a second molecule of the guanidine to form a salt (Scheme 2). A similar outcome was observed when the reaction mixture from treatment of *N*-methylbenzylamine with 2-methy-2-thiolpseudourea sulfate failed to give a solid product. Adjustment of pH with sodium carbonate produced a precipitate, and X-ray crystallography showed that the product was the carbamate salt (not tested).

Biological Evaluation. Effects of the agents were evaluated by daily measuring body weight and food intake of mice fed a diet containing the test compound in comparison with animals

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[a] Abbreviations: AMPK, adenosine monophosphate kinase; DIO, dietary induced obesity; ob/ob, genetically obese mice.

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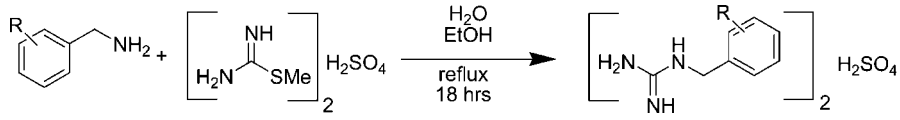
Scheme 1. General Procedure for the Synthesis of Benzylguanidine Sulfates



Scheme 2. Formation of the Benzylguanidine Carbamates

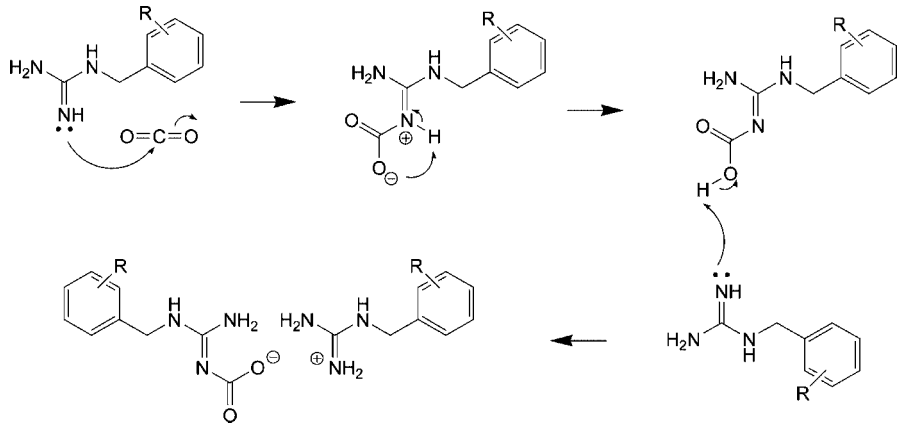


Table 1. Effect Dose of Active Galegine Analogues in BALB/c Mice[a]

compd	dose (mmol/kg body weight/day)	av daily wt difference[b]	ratio of dose/av daily wt difference	av daily difference in food intake[b]	blood glucose at day 7[b]
1	0.50	−16.6 ± 1.7#[c]	0.03	−36.3 ± 1.9#[c]	53 ± 5#[c]
3	0.62	−0.14 ± 0.8NS[c]	4.43	−3.4 ± 2.6NS[c]	106 ± 3NS[c]
14	0.54	−10.4 ± 1.5##[c]	0.038	−28.1 ± 2.0##[c]	60 ± 7##[c]
16	0.72	−2.3 ± 0.4NS[c]	0.31	+5.6 ± 2.8NS[c]	88 ± 7NS[c]
19	0.60	−7.0 ± 1.4NS[c]	0.086	−4.1 ± 8.2NS[c]	NT[c]
20	0.51	−6.0 ± 2.2#[c]	0.085	−17.7 ± 13.9#[c]	84 ± 9NS[c]
21	0.57	−10.7 ± 1.3NS[c]	0.027	−21.1 ± 9.4NS[c]	82 ± 7#[c]
27	0.62	−14.7 ± 1.8#[c]	0.042	−27.8 ± 4.2#[c]	56 ± 3##[c]
28	0.70	−10.6 ± 0.6#[c]	0.066	−12.7 ± 7.5NS[c]	75 ± 2NS[c]
29	0.59	−19.7 ± 1.0#[c]	0.029	−34.7 ± 3.6NS[c]	59 ± 19##[c]
30	0.52	−23.3 ± 0.6#[c]	0.022	−36.7 ± 5.2#[c]	51 ± 1##[c]
33	0.40	−9.9 ± 0.6##[c]	0.04	−38.3 ± 7.4##[c]	67 ± 3NS[c]
34	0.60	−2.0 ± 0.8NS[c]	0.30	−18.0 ± 8.7′	NT[c]

[a] Each compound administered in feed for 7 days. [b] (% from time-matched controls; + SEM). [c] Statistical analyses. Body weight and food intake data analysed (using raw data) by 2-way ANOVA for repeated measures compared with appropriate control. Blood glucose data analysed (using raw data) from samples obtained at day 7 by one way ANOVA followed by Dunnett's post hoc compared with appropriate control. NT = Not tested; NS = not significant; # = <0.05; ## = <0.01.

fed a control diet. A selection of compounds from these studies were examined further in mice fed a high fat diet to produce dietary-induced obesity (DIO) and/or in genetically obese (*ob*/*ob*) mice. The *ob*/*ob* mouse demonstrates the obese phenotype as a consequence of failure to produce leptin,[15] whereas diet-induced obesity may be more representative of typical human obesity in which hyperleptinaemia is present[16] and where excessive calorie intake interacts with particular genetic backgrounds to produce obesity.[17]

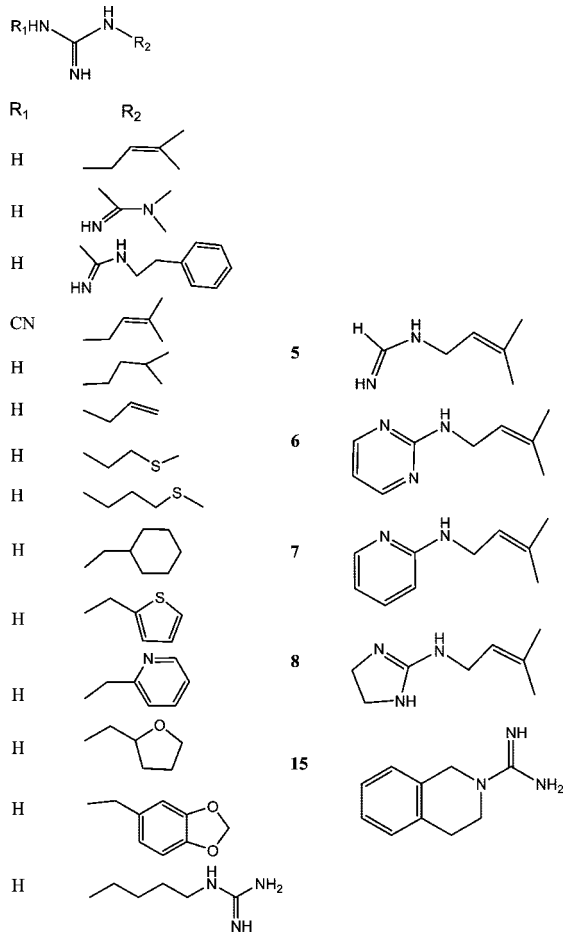
Initial Screening and Structure−Action Relationships. Mice fed on a galegine-containing diet showed less weight gain than control animals over a 7-day test period (Table 1). Treated mice ate less than the control group, particularly in the first two days, but they showed no obvious differences in behavior or overt signs of toxicity. Blood glucose levels determined at the end of the treatment period were reduced in the galegine-fed group (Table 1). There were no consistent changes in blood lactate levels (data not shown).

From a consideration of its physicochemical properties, it is unexpected that **1** should be active by oral administration. Guanidine itself is such a strong base that the pK_a cannot be easily assessed by normal aqueous titration although a value of 13.6 is given.[18] Guanidine is thus permanently protonated in aqueous solution and does not satisfy the normal specification for a "drug-like" molecule, which anticipates that a less polar form of a base can be formed by deprotonation, allowing passage through lipid membranes. To be orally effective, guanidine derivatives must presumably be taken up by an active mechanism or by a carrier. The uptake mechanism may be the same as that used for cationic amino acids, specifically arginine.[19]

We reasoned that it was desirable to find active analogues that would be less strongly basic and that might pass through membranes in a passive manner despite the observation[20] that guanidine-rich peptides have special properties with regard to tissue penetration. With this in mind, we first examined modifications to the guanidine moiety of galegine (Table 2). The in vivo assay was carried out at one dose level for each new compound, the dose chosen being approximately equimolar to an effective dose of galegine (3.41 mmol compound per kg of mouse diet, which gave an average daily dose of galegine of 0.5 mmol/kg body weight).

N-Cyano substitution, as with cimetidine,[21] would be expected to reduce basicity very efficiently, but analogue **4** was inactive in the in vivo assay. Amidines are less strongly basic[22] than guanidines, and the direct galegine analogue **5** was readily prepared but was inactive at the dose level used for **1**.

Table 2. Structures of Galegine and its Non-Benzyl Group Containing Analogues

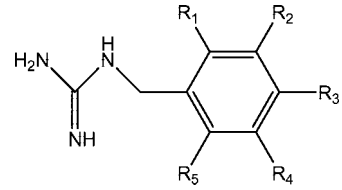


Incorporation of one of the nitrogens into a pyrimidine ring was anticipated to have a marked effect on basicity, given that pyrimidine itself is very weakly basic,[23] but again the analogue **6** was inactive.

Two more heterocyclic analogues were synthesized: the pyridine analogue **7**, in which one nitrogen is in a pyridine ring, and the imidazolidine **8**, which is structurally very close to galegine but neither was active. The latter is particularly surprising and shows that a terminally unmodified guanidine is critical for weight-loss activity in our series of compounds. This was not the case in studies by Vaillencourt and Larsen et al.[24,25] in which they found that small modifications to the guainidine facilitated weight loss. Similar effects were also observed in studies by Tassoni et al.,[26] where modification to the guanidine group resulted in a decrease in food intake. It is significant that phenformin **3** was inactive in the weight-loss assay used here at the chosen dose level, showing that modification of the guanidine to give a biguanide also loses or at least reduces the activity in mice: phenformin has been reported to reduce weight in humans.[27]

Modification to the dimethylallyl group of galegine (Table 2) was first effected with a simple isopentyl chain to give **9**, which lost much of the activity. The allyl analogue **10** had no detectable activity at the dose employed. Replacement of the dimethylallyl moiety by a thioether of similar size and lipophilicity, as in **11** or **12**, resulted in complete loss of activity, as did replacement with cyclohexylmethyl to give **13**. The lack of activity with **13** is consistent with a binding site that can only accommodate flattened molecules in a hydrophobic pocket.

Table 3. Benzyl Group Containing Analogues of Galegine



	R_1	R_2	R_3	R_4	R_5
14	H	H	H	H	H
19	OMe	H	H	H	H
20	Br	H	H	H	H
21	Cl	H	H	H	H
22	Me	H	H	H	H
23	CF3	H	H	H	H
24	Cl	H	H	H	F
25	H	OMe	H	H	H
26	H	H	Me	H	H
27	H	H	F	H	H
28	H	H	CF3	H	H
29	H	H	Cl	H	H
30	H	H	Br	H	H
31	H	H	OMe	H	H
32	H	H	OEt	H	H
33	H	Cl	Cl	H	H
35	H	OMe	OMe	H	H
36	OMe	OMe	H	H	H
37	OMe	H	OMe	H	H
38	OMe	H	H	OMe	H
39	OMe	OMe	OMe	H	H
40	H	H	OH	H	H

Replacement of dimethylallyl by a benzyl substituent was therefore an obvious modification, relatively simple from a synthetic viewpoint, and offering multiple possibilities for further modifications. The simple benzyl compound **14** proved to be similar to galegine in potency (Table 1).

Structural modification of the benzyl group (Table 3) was easier from a synthetic point of view than with the analogous dimethylallyl, so efforts were concentrated on modifications to **14**. Replacement of the benzylamine by tetrahydroisoquinoline to give **15** abolished activity, so further structural changes concentrated on the phenyl ring of **14**. Very surprisingly, replacement of benzyl with the isosteric thiophene to give **16** resulted in complete loss of activity. The pyridine analogue **17** was also inactive, at least at the chosen dose level. The observations with **16** and **17** contrast with the "formin" series, where both the thiophene and pyridine analogues had good activity.[4] The tetrahydrofuran analogue **18** was inactive, consistent with a requirement for planarity at the position β to the guanidine.

Substitution in the *ortho* position reduced potency, as in **19**, **20** and **21**, or abolished activity altogether, as in **22**, **23**, and **24**, yet another indication of the very restrictive steric requirements of the biological binding site. The 3-methoxy analogue **25** was inactive, but *para* substitution gave some very interesting results. While the 4-methyl analogue **26** was inactive, 4-fluoro substitution as in **27** gave an active compound; it is worth noting that this compound and its *meta* isomer were filed for patenting as antidiabetic agents.[28] The 4-trifluoromethyl analogue **28**, the most potent hypotensive agent in a series of benzylguanidines,[11] was moderately active in inducing weight loss in the present work. The 4-chloro analogue **29** was more potent than galegine: *p*-chloro substitution also gave a potent hypoglycemic in the "formin" series.[4] The 4-bromo compound **30** showed activity comparable to that of the 4-chloro analogue **29**. The 4-methoxy and 4-ethoxy analogues **31** and **32** were inactive, perhaps owing to a shape mismatch. In contrast, the 4-methoxy analogue in

Table 4. Effect of Galegine Analogues in *ob*/*ob* Mice[a]

compd	dose (mmol/kg body wt/day)	av daily wt difference[b]	ratio of dose/av daily wt difference	av daily difference in food intake	blood glucose at day 28[b]
1	0.26	-7.3 ± 0.3#[c]	0.037	-29.2 ± 3.0#[c]	91 ± 9NS[c]
14	0.26	-6.8 ± 0.3#[c]	0.038	-29.3 ± 2.6#[c]	82 ± 10NS[c]
27	0.27	-8.7 ± 0.4##[c]	0.031	-26.8 ± 3.3##[c]	102 ± 21NS[c]
29	0.24	-11.0 ± 0.7##[c]	0.022	-38.5 ± 3.2#[c]	71 ± 10NS[c]

[a] Each compound administered in feed for 28 days. [b] (% from time-matched controls; ± SEM). [c] Statistical analyses for data presented. Body weight and food intake data analysed (using raw data) by 2-way ANOVA for repeated measures compared with appropriate control. Blood glucose data analysed (using raw data) from samples obtained at day 28 by one way ANOVA followed by Dunnett's post hoc compared with appropriate control. NT = not tested; NS = not significant; # = <0.05; ## = <0.01.

Table 5. Effect of Galegine Analogues in DIO Mice[a]

compd	dose (mmol/kg body wt/day)	av daily wt difference[b]	ratio of dose/av daily wt difference	av daily difference in food intake[b]	blood glucose at day 28[b]
1	0.38	-9.0 ± 1.0#[c]	0.042	-23.6 ± 2.1##[c]	77 ± 2##[c]
14	0.43	-10.6 ± 0.4NS[c]	0.041	-14.9 ± 3.1NS[c]	90 ± 6NS[c]
27	0.45	-7.8 ± 0.6NS[c]	0.057	-9.8 ± 3.6NS[c]	93 ± 3NS[c]
29	0.42	-7.3 ± 0.8NS[c]	0.058	-15.6 ± 5.2N[c]S	94 ± 4NS[c]

[a] Each compound administered in feed for 28 days. [b] (% from time-matched controls; + SEM). [c] Statistical analyses for data presented. Body weight and food intake data analyzed (using raw data) by 2-way ANOVA for repeated measures compared with appropriate control. Blood glucose data analyzed (using raw data) from samples obtained at day 28 by one-way ANOVA followed by Dunnett's post hoc compared with appropriate control. NT = not tested; NS = not significant; # = <0.05; ## = <0.01.

the "formin" series retained a good level of hypoglycemic activity in guinea pigs.[4]

3,4-Disubstitution as in **33** and **34** resulted in lower activity than galegine or **14**, while 3,4-dimethoxy substitution to give **35** abolished activity. The 2,3-dimethoxy **36**, 2,4-dimethoxy **37**, and 2,5-dimethoxy **38** analogues were also inactive, as was the 3,4,5-trimethoxy derivative **39**.

In view of the apparent requirement of the biological binding site for a nonpolar, lipophilic moiety attached to the guanidine, it was not surprising that the 4-hydroxy analogue **40** was inactive. This is also consistent with the lack of activity of the diguanidino compound **41**.

The major route of metabolism of phenformin is oxidative hydroxylation at the *para* position of the phenyl ring.[29] This was also a source of some of the clinical problems because a small proportion of the population lack the CYP_{450} isoform required for this oxidation; these represented some of the patients who suffered from lactic acidosis as the phenformin accumulated.[30] A possible explanation for the increased activity of some of the *para*-substituted benzylguanidines was blockage of the oxidation, but in practice the unsubstituted benzyl analogue **14** and the thiophene **16** showed no sign of oxidation when treated with freshly prepared mouse liver microsomes although we were able to confirm the hydroxylation of phenformin under the same conditions.

From the initial screening, several analogues with galegine-like activity were found: **14**, **21**, **27**, **29**, and **30**. These caused similar or greater degrees of weight loss as did galegine and/or showed greater weight loss with less effect on food intake than galegine (Table 1). They were further tested on models for obesity using *ob*/*ob* mice or mice fed a high-fat diet.

Effects in Obesity Models. The active analogues **14**, **27**, and **29**, and galegine (**1**) were less effective in *ob*/*ob* mice than in normal BALB/c mice (Table 2). These compounds were also effective in mice with diet-induced obesity, although all appeared to be slightly less effective than in normal BALB/c and *ob*/*ob* mice with the exception of the benzyl analogue **14** (Table 3). Although galegine was hypoglycemic in the diet-induced obese mice, the analogues had little or no effect on blood glucose levels with the exception of **29**, which exhibited slight hypoglycemia in the *ob*/*ob* model (Tables 4 and 5).

Conclusions

Many of the structural modifications to galegine were associated with complete loss of activity in vivo, indicating that the structural requirements for absorption or for pharmacological activity, or both, were restrictive. However, several analogues were found to act similarly to galegine in normal mice, i.e., they reduced weight gain over a seven day period with varying degrees of reduced food intake. Most compounds appeared to lower glucose levels when measured after seven days, but there were no effects on levels of lactate in the blood. In the BALB/c and *ob*/*ob* models used here, the chloro **29** analogue showed greater activity than galegine **1** although it was slightly less active in the DIO model. The fluoro **27** analogue was less active than **1** in the BALB/c and DIO models but should have greater activity in the *ob*/*ob* model.

Although the dose levels (0.2−0.5 mmol/kg body weight) used in this study may appear to be quite high, it should be noted that mice are relatively insensitive[31] to the biguanide "formins"; typically, mice require doses of metformin hydrochloride of about 250 mg/kg (ca. 1.5 mmol/kg), whereas humans are treated at 20−25 mg/kg. It is possible that the present series of guanidines will require much lower doses to be effective in other species, including man. Overall, at least two compounds, **27** and **29**, from the present series seem to be promising candidates for the treatment of obesity. The lack of microsomal oxidation is a considerable advantage over phenformin, as is the potential for greater potency in humans.

Experimental Section

Chemistry. All reagents and solvents were of commercial quality; solvents were dried according to standard procedures when deemed necessary. IR spectra were recorded with a Mattson Genesis series FTIR spectrometer, solid samples were pressed potassium bromide (KBr) discs, and liquid samples were films in sodium chloride (NaCl) discs. Wave frequencies, υ_{max} (cm^{-1}) are quoted for appropriate functional groups. Melting points (mp) were determined on a Stuart Scientific melting point apparatus SMP1 and are in °C. ^1H and ^{13}C NMR spectra were recorded on JEOL EX-270 (270 MHz) spectrometer. The deuterated solvent used is specified for each compound, and chemical shifts are expressed in parts per million. Reactions and column chromatographic separations were followed by thin-layer chromatography using silica gel

(with 254 nm fluorescent indicator). Samples were submitted for elemental analysis on a Perkin-Elmer 2400 Analyzer. C, H, and N were determined simultaneously and halogens and sulfur separately after recrystallization of the organic salts from wate.

1-(3-Methylbut-2-enyl)guanidine Hemisulfate 1 (Galegine) and General Procedure for Synthesis of Guanidine Hemisulfates. A mixture of 4-bromo-2-methyl-2-butene (19.4 g, 1.0 equiv, 130 mmol) and potassium phthalimide (29.8 g, 1.2 equiv, 161 mmol) were suspended in DMF (200 mL) and stirred at 120 °C for 1 h before heating to 160 °C and stirring for a further 18 h. The mixture was poured over ice and washed with dichloroethane (5 × 50 mL), and the organic phases were separated and combined before washing with sodium hydroxide solution (0.1 N) (2 × 100 mL) and water (2 × 50 mL). The organic extracts were separated, dried over anhydrous magnesium sulfate, filtered, and concentrated to leave a crude solid that was crystallized from cold ethanol to give the intermediate 2-(3-methylbut-2-enyl)isoindoline-1,3-dione (25.6 g, 93%) as a white solid; mp 100−101 °C. ^{1}H NMR (D_2O) δ 7.88−7.91 (m, 2H), 7.61−7.70 (m, 2H), 5.31 (m, 1H), 4.31 (d, 2H, J = 7.2 Hz), 1.70 (s, 3H), 1.62 (s, 3H); mp 100−101 °C.

A mixture of 2-(3-methylbut-2-enyl)isoindoline-1,3-dione (10 g, 1.0 equiv, 46.6 mmol), ethanol (100 mL), and hydrazine hydrate (85%) (2.9 mL, 1.2 equiv, 51.1 mmol) were stirred under reflux for 1 h, cooled, hydrochloric acid (1M) (5.2 mL, 1.2 equiv, 51.1 mmol) added, and then refluxed for a further 1 h. The mixture was allowed to cool, filtered, and the residue washed with cold water (100 mL) before reducing the filtrate under vacuum to give the intermediate 3-methylbut-2-en-1-amine hydrochloride (4.4 g, 78%) as a white solid; mp 95−97 °C. ^{1}H NMR (D_2O) δ 6.75 (s, 1H), 6.55 (s, 2H), 5.16 (t, 1H, J = 6.3 Hz), 3.68 (d, 2H, J = 6.3 Hz), 1.71 (s, 3H), 1.63 (s, 3H).

2-Methylthiopseudourea sulfate (6.95 g, 1.0 equiv, 150 mmol) and 3-methylbut-2-en-1-amine (9.1 g, 2.0 equiv, 100 mmol) were dissolved in water (100 mL) and ethanol (100 mL). The mixture was stirred at reflux for 18 h, connected to a series of bleach traps, before cooling and reducing under vacuum to give a white crude solid. The compound was suspended in water (30 mL) and heated until it barely dissolved before allowing to slowly cool upon which a white solid began to form. After allowing the formation of the solid to continue overnight, it was collected by filtration and dried in the oven to give the title compound (5.4 g, 62%) as a white solid; mp 216−218 °C (dec); lit.[30] 214−216 °C (dec). ^{1}H NMR (D_2O): mp 216−218 °C (dec); lit.[30] 214−216 °C (dec); δ 5.32 (m, 1H), 3.78 (d, 2H, J = 6.7 Hz), 1.81 (s, 3H), 1.72 (s, 3H). Anal. ($C_{12}H_{28}N_6O_4S$) C, H, N.

((*Z*)-2-Cyano-1-(3-methylbut-2-enyl)guanidine 4. To a solution of 3-methylbut-2-en-1-amine hydrochloride (2.5 g, 1.0 equiv, 20.55 mmol) dissolved in *n*-butanol (20 mL) was added sodium dicyanamide (1.82 g, 1.0 equiv, 20.5 mmol). The mixture was stirred at 140 °C for 18 h and allowed to cool to ambient before filtering to leave a pale-yellow filtrate and a pale-yellow gum. The liquid was decanted off, and the gum was washed with ethanol (3 × 50 mL). The extracts were collected and reduced under vacuum before leaving in the freezer overnight, which gave the title compound (1.72 g, 55%) as an off white solid; mp 193−195 °C. ^{1}H NMR (D_2O) δ 8.21 (s, 3H), 5.34 (m, 1H), 3.43 (d, 2H, J = 7.0 Hz), 1.70 (s, 3H), 1.62 (s, 3H). Anal. ($C_7H_{12}N_4$): C, H, N.

***N*-(3-Methylbut-2-enyl)formamidine Fumarate 5.** Under an atmosphere of nitrogen, formamidine hydrochloride (0.85 g, 1.0 equiv, 10.6 mmol) and 3-methylbut-2-en-1-amine (0.9 g, 1.0 equiv, 10.6 mmol) were dissolved in anhydrous absolute ethanol (20 mL). The mixture was allowed to stir at ambient temperature for 20 min before stirring for 3 h at reflux. The solution was then reduced under vacuum and placed in a freezer in an attempt to crystallize the product. This proved unsuccessful, and so the compound was basified as described previously to give *N*-(3-methylbut-2-enyl)-formamidine (484 mg, 32%) as a colorless oil. This was then converted to the fumarate salt as described previously; mp 212−216 °C. ^{1}H NMR (D_2O) δ 7.81 (s, 1H), 6.54 (s, 2H), 5.12 (m, 1H), 3.61 (m, 2H), 1.71 (s, 3H), 1.67 (s, 3H). Anal. ($C_{10}H_{16}N_2O_4$): C, H, N.

***N*-(3-Methylbut-2-enyl)pyrimidin-2-amine Fumarate 6.** A solution of ethanol (50 mL), 2-chloropyrimidine (1.21 g, 1.0 equiv, 10.6 mmol), and 3-methylbut-2-en-1-amine (0.9 g, 1.0 equiv, 10.6 mmol) were stirred at reflux for 4 h. The mixture was cooled and concentrated under vacuum to give a pale-yellow oil as *N*-(3-methylbut-2-enyl)pyrimidin-2-amine (1.5 g, 64%). This was then converted to the fumarate salt as described previously; mp 232−234 °C. ^{1}H NMR (D_2O) δ 8.28 (d, 2H, J = 4.9 Hz), 6.58 (s, 2H), 6.51 (t, 1H, J = 4.8 Hz), 5.31 (t, CH, J = 8.1 Hz), 3.97 (d, 2H, J = 8.1 Hz), 1.74 (s, 3H), 1.71 (s, 3H). C, H, N.

***N*-(3-Methylbut-2-enyl)pyridin-2-amine Fumarate 7.** A round-bottom flask was charged with anhydrous toluene (60 mL) before adding sodium amide (1.1 g, 1.0 equiv, 25.0 mmol) and 2-aminopyridine in small portions. The mixture was stirred at reflux for 3 h before cooling, upon which γ,γ-dimethylallylbromide (3.72 g, 1.0 equiv, 25.0 mmol) was added. The mixture was allowed to stir at reflux for a further 3 h before cooling and isolating *N*-(3-methylbut-2-enyl)pyridin-2-amine as a colorless oil (3.1 g, 64%) after column chromatography using DCM:EtOH in a 300:8 ratio. The fumarate salt was prepared by adding fumaric acid (1.0 equiv) to the free base dissolved in acetone and heating for 10 min. After cooling, the precipitate was filtered and collected as the desired product; mp 225−230 °C. ^{1}H NMR (D_2O) δ 7.85 (m, 1H), 7.71 (m, 1H), 6.97 (d, 1H, J = 6.2 Hz), 6.87 (t, 1H, J = 4.3 Hz), 6.73 (s, 2H), 5.32 (m, 1H), 3.93 (d, 2H, J = 4.6 Hz), 1.76 (s, 3H), 1.73 (s, 3H). Anal.($C_{16}H_{20}N_2O_6$): C, H, N.

***N*-(3-Methylbut-2-enyl)-4,5-dihydro-1*H*-imidazol-2-amine Fumarate 8.** A solution of 2-methylthio-2-imidazoline hydriodide (2.58 g, 1.0 equiv, 10.6 mmol) and γ,γ-dimethylallylamine (0.9 g, 1.0 equiv, 10.6 mmol) dissolved in water (50 mL) were stirrred at reflux for 2 h. After cooling, the mixture was concentrated under vacuum and allowed to stand in the freezer to facilitate crystallization. This proved unsuccessful, and thus the oil was basified by washing with sodium hydroxide solution (10%) (3 × 50 mL), and the organic phase was extracted with ether (3 × 50 mL) before separating and drying over anhydrous magnesium sulfate. Filtration of the dried organic fractions followed by evaporation under vacuum gave *N*-(3-methylbut-2-enyl)-4,5-dihydro-1*H*-imidazol-2-amine as a pale-yellow oil (189 mg, 13%). This was then converted to the fumarate salt as previously described; mp 228−230 °C. ^{1}H NMR (D_2O) δ 6.58 (s, 2H), 5.21 (m, 1H), 3.68 (s, 4H), 3.59 (d, 2H, J = 6.9 Hz), 1.75 (s, 3H), 1.67 (s, 3H). Anal. ($C_{12}H_{19}N_3O_4$): C, H, N.

1-(4-Ethoxybenzyl)guanidine Hemisulfate 32. 4-Hydroxybenzaldehyde (18.1 g, 1.0 equiv, 148.7 mmol), ethyl bromide (32.4, 2.0 equiv. 297.3 mmol), and potassium carbonate (24.7 g, 1.2 equiv, 178.4 mmol) were dissolved in water (100 mL) and methanol (100 mL) before stirring at reflux for 18 h. The reaction mixture was allowed to cool, washed with water (100 mL), and the organic phase extracted with ether (3 × 25 mL). The organic phases were combined, washed with NaOH solution (10%) (3 × 25 mL), separated, and dried over anhydrous magnesium sulfate before filtering and evaporating under vacuum to afford 4-ethoxybenzaldehyde as a colorless oil (8.4 g, 37.7%). Analysis of the product by TLC, using chloroform as eluent, showed a single spot on the TLC plate (R_f = 0.64). and the compound was used in the next step without purification or further analysis.

Hydroxylammonium chloride (4.5 g, 1.2 equiv, 65.5 mmol) dissolved in water (14 mL) was added dropwise to a stirring solution of 4-ethoxybenzaldehyde (8.2 g, 1.0 equiv, 54.6 mmol) and sodium carbonate (11.6 g, 2.0 equiv, 109.2 mmol) dissolved in water (116 mL) and ethanol (100 mL). The reaction mixture was stirred for 4 h before cooling in an ice bath to furnish a crop of colorless crystals, which were filtered and dried to afford 4-ethoxybenzaldoxime (6.4 g, 77.1%). Analysis of the product by TLC ($CHCl_3$) showed a single spot on the TLC plate (R_f = 0.02), and the compound was used in the next step without purification or further analysis.

4-Ethoxybenzaldoxime (6.3 g, 1 equiv, 38.0 mmol) was dissolved in ethanol (200 mL) and NaOH (10%, 200 mL) before cautiously adding nickel aluminum alloy (9.13 g) portionwise as hydrogen was evolved. After completion of the addition, the mixture was

stirred for 4 h before filtering through kieselguhr, the filtrate evaporated under reduced pressure, and chloroform (40 mL) added. The mixture was washed with brine (3 × 50 mL), the organic phase separated, dried over anhydrous magnesium sulfate, filtered, and evaporated under vacuum to afford 4-ethoxybenzylamine as a pale-yellow oil (3.2 g, 51.1%), which was used immediately in the next reaction step without further purification and the compound used in the next step without purification or further analysis.

Using the general procedure the title compound (9.00 g, 31.5%) was obtained as a white solid; mp 226−227 °C. ^{1}H NMR (D_2O) δ 7.50−7.25 (m, 2H), 7.05−6.93 (m, 2H), 4.43 (s, 2H), 4.10 (q, 2H, J = 7.0 Hz), 1.36 (t, 3H, J = 7.0 Hz). Anal. ($C_{20}H_{32}N_6SO_6$): C, H, N; S.

4-Hydroxybenzylguanidine Hemisulfate 40. Pyridinium toluene-*p*-sulfonate (2.0 g) was added to a stirring solution of 3,4-dihydro-(*2H)*-pyran (68.0 g, 4.8 equiv, 808.4 mmol) in toluene (150 mL) before adding 4-cyanophenol (20.0 g, 1 equiv, 167.9 mmol) and stirring the mixture at reflux for 18 h. After cooling the mixture was washed with potassium carbonate solution (15%) (3 × 100 mL), the organic phase separated, dried over anhydrous magnesium sulfate, filtered, and the solvent evaporated under vacuum. The crude solid product obtained was recrystallized from absolute ethanol to afford 4-tetrahydropyranyloxybenzonitrile as a white solid (17.8 g, 52.5%). Analysis of the product by TLC using chloroform and methanol (9:1) showed a single spot on the TLC plate (R_f = 0.86). The product was used in the next step without further analysis or purification.

A solution of 4-tetrahydropyranyloxybenzonitrile (17.0 g) dissolved in dry ether (40 mL) was added dropwise to a stirring suspension of $LiAlH_4$ (6.0 g) in dry ether (150 mL) and heated at reflux for 1.5 h. After cooling, a fresh solution of saturated sodium sulfite (100 mL) was added dropwise with care before washing with ethyl acetate (3 × 100 mL) and water (3 × 100 mL). The organic phase was separated, dried over anhydrous magnesium sulfate, filtered, and reduced under vacuum to afford 4-tetrahydropyranyloxybenzylamine as a pale-yellow oil (16.4 g, 94.6%). The product was used in the next step without further analysis or purification. Using the general procedure with 4-tetrahydropyranyloxybenzylamine and facilitated by in situ deprotection, the title compound (9.00 g, 26.4%) was obtained as a white solid; mp 268−269 °C. ^{1}H NMR (D_2O): δ 9.56 (s, H), 8.68 (s, 2H), 7.68 (s, H), 7.12 (d, 2H, J = 6.8 Hz), 6.76 (d, 2H, J = 6.8 Hz), 4.10 (s, 2H), 3.36 (s, 1H). Anal. ($C_{16}H_{24}$ N_6SO_6): C, H, N; S.

In Vivo Study Methods. Adult male BALB/c mice were obtained from stock at the Biological Procedures Unit within the University of Strathclyde. A diet-induced obesity (DIO) mouse model was developed by feeding BALB/c mice on a synthetic high-fat (45 kcal%) diet (Research Diets, Inc., New Brunswick, NJ) postweaning through to start of experimental procedures. Homozygous obese *ob*/*ob* mice were purchased from Harlan UK (Bicester, UK). All animals were housed individually in an air-conditioned environment maintained at 21 ± 2 °C with a 12 h light/12 h dark cycle. Animals were allowed continuous access to tap water and unless indicated otherwise were fed ad libitum on standard pellet diet (SDS, Cambridge, UK). Before the initiation of feeding studies, mice were habituated to being housed individually and had their food intake and bodyweight monitored daily at 09.30.

Feeding Studies with Galegine and Analogues Using Normal Mice. These studies investigated the effect of the incorporation into the diet of galegine and synthetic galegine derivatives on daily food intake and bodyweight of lean normal mice over a 7-day test period. At the start of the experimental period (day 0), mice were randomly divided into groups (n = 3) and food removed and substituted with standard diet pellets containing 3.41mmol compound/kg of feed. Daily food intake and bodyweight measurements were recorded for the duration of the 7-day test period, and blood glucose and lactate determinations were made at the end of the study. Blood glucose levels were determined using an Esprit 2 glucometer (Bayer Healthcare, Newbury, UK) and blood lactate concentrations with the use of an Accutrend lactate analyzer (Roche Diagnostics, Mannheim, Germany).

Feeding Studies with Galegine and Analogues Using DIO Mice. These studies investigated the effect of the incorporation into the diet of galegine and synthetic galegine derivatives on daily food intake and bodyweight of mice that had developed an obese phenotype through feeding on a high fat diet for 42 days. At the start of the experimental period (Day 0), mice were randomly divided into groups (n = 6−8) and food removed and substituted with standard diet pellets containing 3.41mmol test compound/kg feed. Daily food intake and bodyweight measurements were recorded for the duration of the 28-day study period, and blood glucose and lactate determinations were made at the end of the study.

Feeding Studies with Galegine and Synthetic Derivatives Using *ob*/*ob* Mice. Male 10-week old *ob*/*ob* mice (n = 6−8) had their feed removed and substituted at day 0 with standard diet pellets containing 2.27 mmol test compound/kg feed. Daily food intake and bodyweight measurements were recorded for the duration of the study period of 28 days. Blood glucose and lactate determinations were made at the end of the study period.

Oxidative Metabolism of 3, 14, 16, and 29. Preparation of Hepatic Microsomes. Livers were removed from four male *ob*/*ob* mice immediately after they were sacrificed by cervical dislocation. Hepatic microsomes were prepared by differential centrifugation as described previously.[40] The microsomal cytochrome P450 (0.287 nM per mg of protein) and protein (19.73 mg/mL) contents were determined by the methods of Watts et al.[41] and Lowry et al.,[42] respectively.

In Vitro Metabolism. Substrates (**3**, **14**, **16**, and **29**) were accurately weighed and made up in deionized water to produce separate 5 mM stock solutions. The microsomal incubation mixture (final volume 1 mL) contained microsomal protein (1 mg), substrate (50 μM, 1:100 dilution of stock), $MgCl_2$ (10 mM) phosphate buffer (100 mM, pH 7.4), and NADPH (8.33 mg/mL). Incubations without NADPH or substrate were used as negative controls. An incubation containing phenformin **3** (50 μM) was used as a positive control. After a 45 min incubation at 37 °C, perchloric acid (70%, 50 μL) was added to the incubation mixture and centrifuged at 4500 rpm (10 min). The supernatant was analyzed by HPLC-MS.

HPLC-MS Analysis. Full scan and selected ion monitoring data (SIM) were obtained with an Agilent 1050 liquid chromatograph coupled to an Agilent LCMSD fitted with an electrospray source. Metabolites and standards were eluted from a Thermo BDS Hypersil C18 column (100 mm × 2.1 mm i.d., 3 μm) at room temperature using a stepwise gradient program of: T = 0 min, 5% v/v MeCN in $NH_4^+CH_3COO^-$ (25 mM, pH 4.0); T = 5 min, 5% v/v MeCN in $NH_4^+CH_3COO^-$ (25 mM, pH 4.0); T = 20 min, 50% v/v MeCN in $NH_4^+CH_3COO^-$ (25 mM, pH 4.0); T = 25 min, 50% v/v MeCN in $NH_4^+CH_3COO^-$ (25 mM, pH 4.0). Nitrogen was used as the nebulizing and drying gas. The capillary voltage was 3 kV; source temperature 150 °C; cone voltage 50 V. Full scanning mass spectra were acquired between m/z 120−280 at 0.93 s/scan. SIM data were acquired for the substrates ($[M + H]^+$) and the specific oxidative transformation [M + (O)] with an average dwell time of 289 ms. Phenformin (tR, m/z 206 for $[M + H]^+$) was metabolized to hydroxyphenformin[43] (m/z 222 for $[M + H]^+$). Compounds **14**, **16**, and **29** showed strong ESI signals for the parent compounds. However, there were no secondary peaks in the LC-MS chromatograms (full scanning mode) indicative of oxidative metabolism. Also, the monitoring of the (M + [O]) biotransformation did not produce evidence of oxidation analogous to the formation of hydroxyphenformin.

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Supporting Information Available: Elemental analyses and crystallographic data for listed for key compounds. Experimental

details listed for all compounds prepared. This material is available free of charge via the Internet at http://pubs.acs.org.

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