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A facile synthesis of Guanyl Hydrazones: Potent Advance Glycation End products' (AGE) inhibitors

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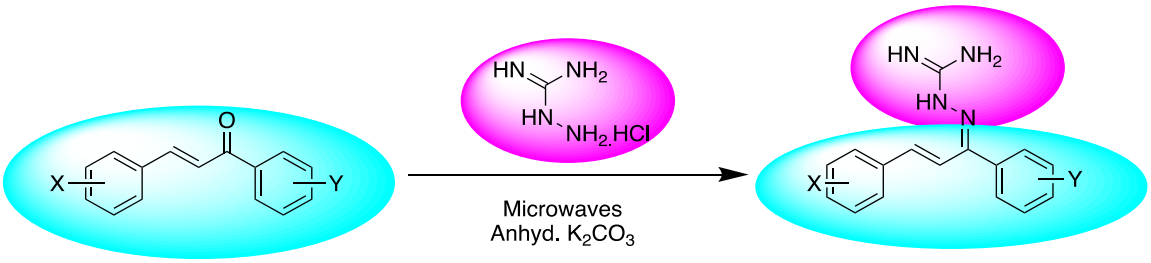
ABSTRACT



A scaffold of potential Advance Glycation End products (AGE) inhibitor, guanyl hydrazones substituted with different functional groups, has been synthesized by facilitating a condensation reaction between differently substituted chalcones and guanyl hydrazone hydrochloride under microwaves, using anhydrous K_2CO_3 as green solid support. The free hydrazones were obtained in high yields through an aqueous post-reaction work-up eliminating the use of organic solvent as well as an external base. Hence an efficient and environmentally benign protocol was developed for the synthesis of the target compounds.

Keywords: Chalcone, aminoguanidine hydrochloride, Guanylhydrazone, microwave irradiation, AGE

INTRODUCTION

The molecular clubbing approach belongs to the most formidable and promising tools in medicinal chemistry and drug design. The combination of various bioactive scaffolds in one template offers several advantages.[1] Aminoguanidine works as an antioxidant by inhibiting selectively inducible nitric oxide synthase and scavenging reactive oxygen species, thus protecting various cells and tissues from oxidative stress. Aminoguanidine also plays an important role as the inhibitor of the **advanced glycation pathway** in the pathogenesis of late diabetes mellitus complications like angiopathy, neuropathy, nephropathy, etc. It is able to scavenge carbonyl reactive intermediates.[2,3] Aminoguanidine and its derivatives also show multiple anti-inflammatory properties.[4–6] But the therapeutical potential is obliterated due to nucleophilic reaction with pyridoxal phosphate (the active form of vitamin B6) leading to its depletion and in vivo deficiency. It has been reported that the formation of aminoguanidine hydrazones by blocking the nucleophilic activity, helps overcome this in vivo deficiency,[3] and establishes these compounds as potent AGE (advanced glycation end products) inhibitors,[7] The structure-activity relationship has established that the presence of a double bond with conjugation is responsible for the biological activities.[8] Therefore the chalcones, consisting of three carbon, α,β-unsaturated carbonyl system,[9] were chosen for hydrazone formation,[10] anticipating a further boost in the pharmacological prospects of aminoguanidine. A wide spectrum of biological activities such as antibacterial, antifungal, antileishmanial, anticonvulsant,[11] antimalarial, antiviral, anticancer, anti-inflammatory, and antioxidant[12,13] is associated with chalcones. So, the clubbing of aminoguanidine with chalcones bringing together two moieties of very high medicinal value was facilitated to obtain a scaffold of aromatic guanyl hydrazones.

The protocol reported here is a direct and simple method where differently substituted chalcones are reacted with aminoguanidine hydrochloride under the microwaves on anhydrous K_2CO_3 solid

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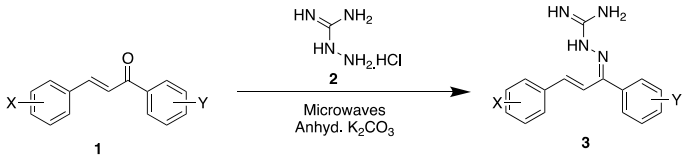
support. K_2CO_3 being water soluble enables an aqueous post-reaction work-up thus minimizing the use of organic solvents, being basic in nature it also furnishes free guanylhydrazone as the final product with higher yields than the reported conventional,[14,15.16] and microwave-assisted protocols.[17]

RESULT AND DISCUSSION

The main objective of this work is to develop a quick and efficient method for the synthesis of guanylhydrazones by condensation of chalcones with aminoguanidine hydrochloride, targeting elimination of organic solvents and harsh external base along with the reduction in reaction times and improvement in product yields. The conventional synthesis of guanylhydrazones by condensation of aminoguanidine hydrochloride with a carbonyl compound in ethanol, refluxing with an acid catalyst suffers from ***long reaction times, low yields,*** and the formation of the ***hydrochloride of guanylhydrazone*** which needs to be transformed[18] into the corresponding free base by treatment with aq. KOH. This is the major drawback associated with all reported methods. Microwave irradiation (MWI) using commercial domestic ovens has been reported to accelerate organic reactions, leading to a significant reduction in the time of reaction and enhanced yields.[19,20] The prior reports of neat synthesis[17] of guanyl hydrazones under microwaves confirm yield enhancement and a reduction in reaction time but led to the hydrochloride of guanyl hydrazone product which needs to be converted to free base, eventually. The protocol reported here is better and greener, and efficiently overcomes all these drawbacks. The reaction between **(1a)** and **(2) scheme1,** as a model reaction was first performed over basic alumina (inorganic solid support) under microwaves which could afford the free guanylhydrazone as the final product but the yield was poor than the neat reported method[17] because the product binds very tightly to the support thus leading to observed lower product yield. To overcome this problem, we decided to use anhydrous K_2CO_3 as a green solid support. The reason behind choosing K_2CO_3 as solid support was to exploit its water-soluble nature for an aqueous workup and basic nature to eliminate the use of an external base. After the completion of the reaction, the reaction mixture was subjected to an aqueous work-up, K_2CO_3 being soluble in water completely dissolved and the product being insoluble was separated by filtration. The yield was much higher than with the basic alumina as shown in (**Table 1)** this can be attributed to the fact that the product could not bind with the support and hence there was no unnecessary product loss. Finding **method B** to be superior the target compounds were synthesized by using K_2CO_3 as solid support. The method worked well with differently substituted chalcones[21,22] **(Scheme 1)** and all the target compounds were obtained in good yields. The product structures were characterized with the help of spectral data (^{1}H NMR, IR, and mass) and elemental analysis. The observed data was consistent with the proposed structure., The ^{1}H NMR showed a broad singlet between 4.93-5.23 ppm and the relative intensity accounted for four hydrogens attached to nitrogen, which could be attributed to the rapid equilibrium between two tautomeric forms **(Figure 1)** structures **(A)** and **(B)**. The geometry of the carbon-carbon double bond in the product structure was found to be trans **(Figure 2)** structure **(C)** as the coupling constant *J* for two vinylic protons was between 12-14 Hz, as evident in the ^{1}H NMR spectrum. The IR spectra of the products showed bands between 1683-1631 cm^{-1} due to the C=N stretch. Also appeared, the broad bands between 3400-3160 cm^{-1} due to NH stretch. The CHN data and m/z molecular ion peak further supported the product structures.



Compd. No.	3a	3b	3c	3d	3e	3f	3g
X	H	4-CH_3O	4-Cl	3-NO_2	4-CH_3O	3,4-(OCH_2O)	H
Y	H	H	H	H	4-Cl	4-Br	4-Cl

Scheme 1. Synthesis of Guanyl hydrazones (3a-g) from chalcone (1a-g) and aminoguanidine hydrochloride (2)

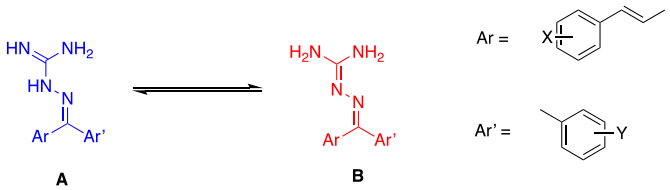


Figure 1. Tautomeric structures of guanyl hydrazones

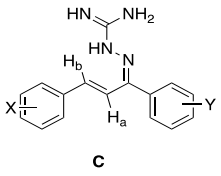


Figure 2. Structure showing stereochemistry of C=C

EXPERIMENTAL

Microwave reactions were carried out in Kenstar Microwave Oven, Model No. OM9925E (2450 MHz, 800 W) and IR spectra were recorded on a Perkin Elmer FTIR-1710 spectrophotometer using Nujol film. IR frequency is measured in cm^{-1}.^{1}H NMR spectra were recorded on NMR Bruker Advance (300 MHz) Spectrometer. The chemical shifts, δ, are given in ppm, relative to internal reference, tetramethylsilane (TMS), with the notations, s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, and brs-broad singlet. Elemental analyses were performed using Heraeus CHN-Rapid Analyser. EI mass spectra were taken on KC 455 Waters TOF MS spectrometer. The melting points were determined on Thomas Hoover melting point apparatus and are uncorrected. The purity of the compounds was checked through TLC on Aluminium plates coated with silica gel (Merck).

General procedure for the synthesis of 3a-g under microwaves using basic alumina as solid support

Method A: To an ethanolic solution of chalcone **(1)** (1 mmol) and aminoguanidine hydrochloride **(2)** (2 mmol) 20 g of basic alumina was added with stirring. The reaction mixture was stirred, air-dried, and irradiated in a domestic microwave oven (800 W) at an interval of 1 min. Upon completion of the reaction as monitored by TLC the reaction mixture was eluted with ethanol which on evaporation gave the desired product **(3)** which was recrystallized with aqueous ethanol.

General procedure for the synthesis of 3a-g under microwaves using anhydrous K_2CO_3 as solid support

Method B: To an ethanolic solution of chalcone **(1) (**1mmol) and aminoguanidine hydrochloride **(2)** (2mmol) 15 g of anhydrous K_2CO_3 was added with stirring. The reaction mixture was stirred, air-dried, and irradiated in a domestic microwave oven (800 W) at an interval of 1 min. Upon completion of the reaction as monitored by TLC the reaction mixture was treated with water. The K_2CO_3 dissolved in the water and the crude product **(3)** was separated by filtration dried and recrystallized from aqueous ethanol.

Table 1: Comparison of Reaction Time/Yield of Compounds (3a-g)

Compd. No.	X	Y	Microwave		M.P. °C
			Method A Time (min)/ Yield (%)	Method B Time (min)/ Yield (%)	
3a	H	H	6.5/75	3.5/82	94-96
3b	4-CH_3O	H	6.0/78	3.5/84	86-87
3c	4-Cl	H	7.0/82	4.0/89	116-118
3d	3-NO_2	H	8.0/85	2.5/90	108-110
3e	4-CH_3O	4-Cl	9.0/87	3.0/92	102-105
3f	3,4-(OCH_2O)	4-Br	9.5/86	4.0/90	115-117
3g	H	4-Cl	7.0/82	4.0/90	121-123

(*E*)-2-((*E*)-1,3-diphenylallylidene) hydrazine-1 carboximidamide (**3a**): M.P.: 94-96°C; IR (nujol) 3312, 1610, 1597 cm^{-1}; 1H NMR (300 MHz, $CDCl_3$): 5.1 (brs, 4H, NH), 5.91 (d, *J* = 18 Hz, 1H, H_a), 6.59 (d, *J* = 13Hz, 1H, H_b), 7.12-7.53 (m, 10H, Ar-H); Anal. Calc. for $C_{16}H_{16}N_4$; C 72.70% H 6.10% N 21.20%, Found: C 71.93%; H 6.02%; N 20.01% m/z: 264.1343

(*E*)-2-((*E*)-3-(4-methoxyphenyl)-1-phenylallylidene) hydrazine-1-carboximidamide (**3b**): M.P.: 86-87°C; IR (nujol) 3350, 1598, 1591 cm^{-1}; 1H NMR (300 MHz, $CDCl_3$): δ 3.81 (s, 3H, OCH_3), 4.93 (brs, 4H, NH_2), 5.42 (d, *J* = 16Hz, 1H, H_a), 6.59 (d, *J* = 13Hz, 1H, H_b), 7.01-7.49 (m, 9H, Ar-H) Anal. Calc. for $C_{17}H_{18}N_4O$: C 69.37% H 6.16% N 19.03% Found: C 68.96%; H 5.89%; N 18.57%.m/z: 294.1432.

(*E*)-2-((*E*)-1-(4-chlorophenyl)-3-phenylallylidene) hydrazine-1-carboximidamide (**3c**): M.P.: 116-118°C; IR (nujol) 3310, 1695, 1595 cm^{-1}; 1H NMR (300 MHz, $CDCl_3$): 5.11 (brs, 4H, NH_2), 5.89 (d, *J* = 18 Hz, 1H, H_a), 6.51 (d, *J* = 12 Hz, 1H, H_b) 6.99-7.41 (m, 9H, Ar-H) Anal. Calc. for $C_{16}H_{15}ClN_4$: C 64.32% H 5.06% N, 18.75%, Found: C 63.24%; H 4.67%; N 18.12%.m/z: 298.0985

(*E*)-2-((*E*)-3-(3-nitrophenyl)-1-phenylallylidene) hydrazine-1-carboximidamide (**3d**): M.P.: 108-110°C; IR (nujol) 3310, 1695, 1595 cm^{-1}; 1H NMR (300 MHz, $CDCl_3$) : 5.23 (brs, 4H, NH_2), 5.62 (d, *J* = 18 Hz, 1H, H_a), 6.34 (d, *J* = 12 Hz, 1H, H_b), 7.29-7.91 (m, 9H, Ar-H)Anal. Calc. for $C_{16}H_{15}N_5O_2$: C 62.13% H 4.89% N 22.64%, Found: C 61.34%; H 3.92%; N 21.76%. m/z: 309.1213.

(*E*)-2-((*E*)-1-(4-chlorophenyl)-3-(4-methoxyphenyl)allylidene) hydrazine-1-carboximidamide (**3e**): M.P.: 102-105°C; IR (nujol): 3340, 1685, 1595 cm^{-1}; 1H NMR (300 MHz, $CDCl_3$): 5.21 (brs, 4H, NH_2), 5.61 (d, *J* = 16 Hz, 1H, H_a), 6.32 (d, *J* = 12 Hz, 1H, H_b), 7.01-7.34 (m, 8H, Ar-H); Anal. Calc. for $C_{17}H_{17}ClN_4O$: C 62.10% H 5.21% N 17.04%, found C 61.23% H 4.98% N 16.79%, m/z: 328.0102.

(*E*)-2-((*E*)-3-(benzo [*d*] [1,3] dioxol-5-yl)-1-(4-bromophenyl) allylidene) hydrazine-carboximidamide (**3f**): M.P.: 115-117°C; IR (nujol) 3310, 1595, 1586 cm^{-1}; 1H NMR (300 MHz, $CDCl_3$) : 5.23 (brs, 4H, NH_2), 5.9 (s, 2H, OCH_2O), 5.62 (d, *J* = 16 Hz, 1H, H_a), 6.34 (d, *J* = 13 Hz, 1H, H_b), 6.73-7.32 (m, 7H, Ar-H)Anal. Calc. for $C_{17}H_{15}BrN_4O_2$: C 52.73% H 3.90% N 14.47%, Found: C 51.23% H 3.49%; N 13.53%. m/z: 385.0964

(*E*)-2-((*E*)-3-(4-chlorophenyl)-1-phenylallylidene)hydrazine-1-carboximidamide (**3g**): M.P.: 121-123°C; IR (nujol) 3340, 1655, 1595 cm^{-1}; 1H NMR (300 MHz, $CDCl_3$): 5.12 (brs, 4H, NH_2), 5.89 (d, *J* = 17 Hz, 1H, H_a), 6.51 (d, *J* = 12 Hz, 1H, H_b), 6.99-7.40 (m, 9H, Ar-H) Anal. Calc. for $C_{16}H_{15}ClN_4$: C 64.32% H 5.06% N 18.75%, Found: C 63.63%; H 4.76%; N 18.21%.m/z: 298.0783.

Conclusion

A microwave-assisted green protocol for the synthesis of biologically important guanyl hydrazones has been developed, this protocol eliminates the need for organic solvents and external bases. High yields, short reaction times, and an aqueous post-reaction work-up are the added advantages. This protocol is consistent with green chemistry principles for environmentally benign organic synthesis methods. The compounds thus synthesized may come out to be promising AGE inhibitors.

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Conflict of Interest Statement

The author does not have any conflict of interest for this work.

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