Bioorganic & Medicinal Chemistry Letters 23 (2013) 5275–5278

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Evaluation of the Influence of thiosemicarbazone–triazole hybrids on genes implicated in lipid oxidation and accumulation as potential anti-obesity agents

Henok H. Kinfe [a,*], Yonas H. Belay [a], Jitcy S. Joseph [b], Emmanuel Mukwevho [b,*]

[a] Department of Chemistry, University of Johannesburg, PO Box 524, Auckland park 2006, South Africa
[b] Department of Biochemistry, University of Johannesburg, PO Box 524, Auckland Park 2006, South Africa

ARTICLE INFO

Article history:
Received 31 May 2013
Revised 1 August 2013
Accepted 5 August 2013
Available online 13 August 2013

Keywords:
Thiosemicarbazon–triazole hybrid
Obesity
Diabetics
Lipid

ABSTRACT

A series of thiosemicarbazone–triazole hybrids **1a**–**h** are efficiently synthesised and evaluated for their influence on the expression of genes, *cpt-1*, *acc-1* and *pgc-1*, which are essential in lipid metabolism. The test results show that hybrids **1c** and **1g** exhibited relatively high influence on the expression of *cpt-1* and *pgc-1* and suppression of *acc-1* as desired.

Obesity is a serious chronic disease that may lead to type 2 diabetes and insulin resistance.[1] Impaired lipid metabolism and diet (e.g., high calorie diet such as fast foods, fizzy drinks, beacon etc) contribute significantly to lipid accumulation in the body. It is well established that excess accumulation of lipids/fats in adipose and intramuscular tissues results in obesity which predisposes individuals to metabolic diseases such as type 2 diabetes.[2,3] Lipid metabolism occurs mainly in the mitochondria and is controlled by a set of mitochondrial enzymes responsible for both lipid oxidation and synthesis. For instance, carnitine palmityol transferase (CPT)-1 enzyme encoded by *cpt-1* gene controls the rate limiting step in the mitochondrial lipid oxidation while, on the contrary, mitochondrial acetyl-CoA carboxylase (ACC)-1 enzyme encoded by *acc-1* gene catalyzes lipid synthesis and accumulation by inhibiting CPT-1. Literature reports have indicated that down-regulation of ACC-1 and up-regulation of CPT-1 may result in increased oxidative capacity of the mitochondria by reducing lipid accumulation and obesity in adipocytes and myocytes.[4–21] The essential roles of ACC-1 and CPT-1 in regulating lipid synthesis and oxidation, respectively, makes them important targets for the development of effective therapeutic modalities that can treat or better manage obesity and type 2 diabetes.

In our pursuit in the identification of novel hybrid compounds, which comprise two or more drug pharmacophores in one molecule with the intention to exert multi-drug action,[22] we are interested in thiosemicarbazone and triazole hybrids and evaluation of their biological activities. Thiosemicarbazones having several metal binding sites possess a wide spectrum of biological activity which includes antiviral,[23] anticancer[24] and antimalaria[25] activities probably mediated via chelation to intracellular cations. Equally important are triazoles which can interact with biological targets through hydrogen bonding and dipole interactions.[26] Besides their wide spectrum of biological activities,[27–30] they have also exhibited anti-obesity benefits.[31] In this regard, our group has recently reported the synthesis of novel thiosemicarbazone and triazole hybrids **1a**–**h** (Fig. 1) and their antimalarial activity.[32] Furthermore, the anti-obesity activity of triazole derivatives and the bioisosterism of thiosemicarbazones to metformin **2** (Fig. 1), an oral antidiabetic drug which is believed to inactivate *acc-1* inhibiting fatty acid synthesis and stimulating fatty acid oxidation,[33–35] prompted us to investigate the synergic influence of the hybrids on the gene expression of *cpt-1* and suppression of *acc-1*. Herein, we report the synthesis of thiosemicarbazone-triazole hybrids **1a**–**h** and their effect on the expression and suppression of the essential genes, *cpt-1* and *acc-1*, respectively.

The hybrids **1a**–**h** were synthesized according to the recently reported protocol as outlined in Scheme 1.[32] The synthesis commenced with alkylation of commercially available 4-hydroxybenzaldehyde with propargyl bromide in the presence of K_2CO_3 to give

* Corresponding authors. Tel.: +27 11 559 3918; fax: +27 11 5592819.
E-mail addresses: hhkinfe@uj.ac.za (H.H. Kinfe), emukwevho@uj.ac.za (E. Mukwevho).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.08.028

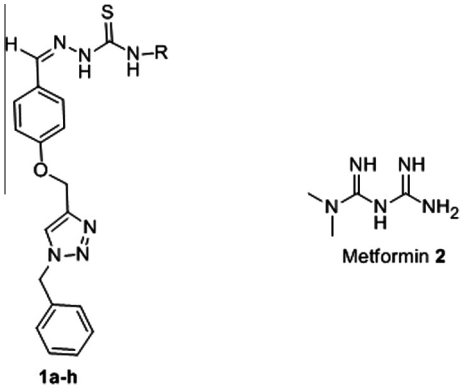


Figure 1. The general structure of the thiosemicarbazone–triazole hybrids (**1a**–**h**) and metformin (**2**).

4 which incorporates an alkynyl group required for click chemistry. Compound **4** was then subjected to click chemistry with freshly prepared benzyl azide to provide 1,4-disubstituted triazole **5**. Methylhydrazinecarbodithioate **7**, which was prepared in a one pot synthesis from the condensation of hydrazine monohydrate, CS_2 and methyl iodide, reacted with triazole **5** under Schiff's base condensation reaction conditions to give compound **8**. The latter then underwent nucleophilic substitution reactions with a series of primary amines to provide a library of hybrid compounds **1a**–**h** as shown in Table 1.

After successful synthesis, hybrids **1a**–**h** were investigated for their effect on *cpt-1* gene expression on 3T3-L1 adipocytes using quantitative real time PCR (qPCR).[36] The expression of *cpt-1* in relation to the test compounds was compared with that of insulin as a standard drug that induces much of mitochondrial gene expression including *cpt-1*. The results are reported in terms of expression ratio and are summarized in Figure 2. Unfortunately, upon treatment with **1a** at different concentrations, the cells lifted from the plate which might be due to toxicity, hence, they could not be used for RNA extraction. Interestingly, similar responses were not observed with the other hybrid compounds. It is evident from the data that **1b**, **1c**, **1d**, **1e** and **1g** exhibited high expression of *cpt-1* relative to

Table 1
Synthesized thiosemicarbazone–triazole hybrids and their yields

Entry	RNH_2	Product	Yield (%)
1	$PhCH_2NH_2$	**1a**	72
2	$CH_3CH_2CH_2CH_2CH_2NH_2$	**1b**	80
3	$PhCH_2CH_2NH_2$	**1c**	74
4	$(CH_3)_2NCH_2CH_2NH_2$	**1d**	78
5	$HOCH_2CH_2CH_2CH_2CH_2CH_2NH_2$	**1e**	66
6	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2NH_2$	**1f**	85
7	$CH_3CH(CH_3)CH_2NH_2$	**1g**	70
8	$HOCH_2CH_2NH_2$	**1h**	62

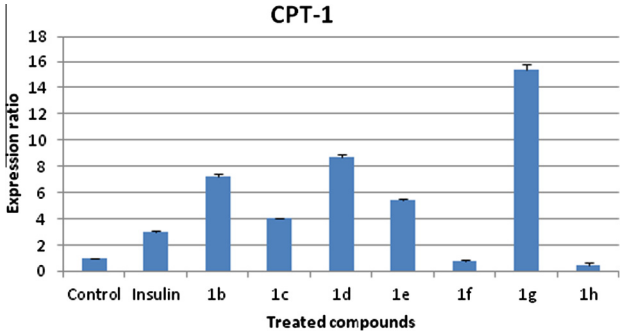
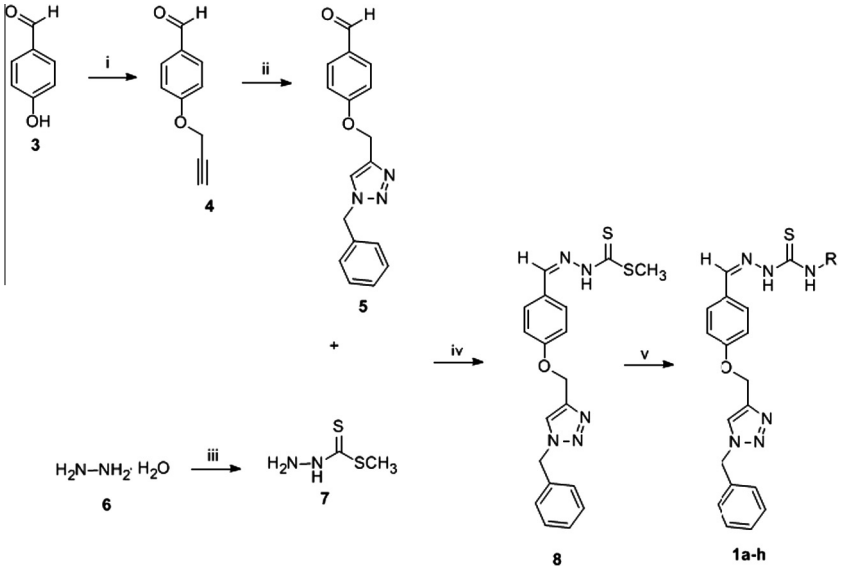


Figure 2. *Cpt-1* gene expression in response to treatment with hybrids **1b**–**h**.

insulin and the control. **1g** showed a more pronounced increase (fivefold of the insulin) followed by **1d** and **1b** with three and two folds, respectively. On the contrary, **1f** and **1h** exhibited suppression of *cpt-1*.

Hybrids **1b**–**h** were further investigated if they can suppress the *acc-1* gene. Interestingly, all except **1h** suppressed the *acc-1* (Fig. 3). The effect of **1f** was almost fourfold lower than that of the control and insulin while **1c**, **1d** and **1g** displayed a twofold suppression.

The results suggest that **1c**, **1d** and **1g** containing short branched chain are potent stimulators of *cpt-1* and inactivators of *acc-1*. To investigate the consistency of **1c**, **1d** and **1g** towards pro-lipid oxidation, a further test was conducted. The gene selected



Scheme 1. Reagents and conditions: (i) K_2CO_3, propargyl bromide, acetone, reflux, 2.5 h, 92%; (ii) BnN_3, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMF:H_2O (4:1), 60 °C, 3 h, 80%; (iii) CS_2, KOH, CH_3I, H_2O:isopropanol (1:1), rt, 4 h, 90%; (iv) **5**, **7**, MeOH, reflux, overnight, 89%; (v) RNH_2, MeOH, reflux, 24 h, 62–85%.

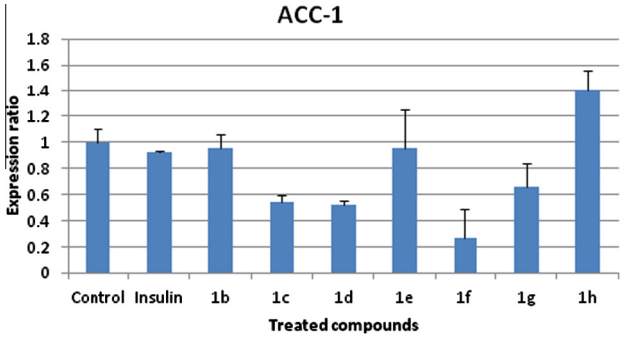


Figure 3. *Acc-1* gene suppression in response to treatment with hybrids **1b–h**.

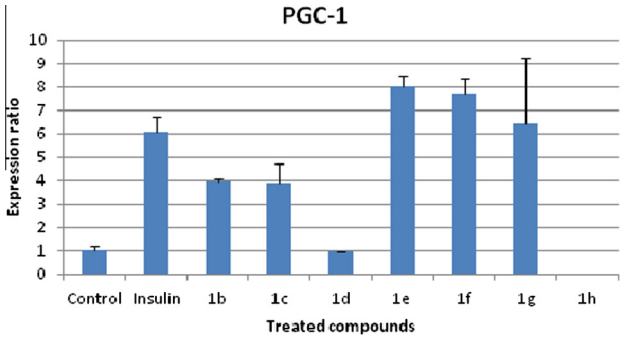


Figure 4. *Pgc-1* gene expression in response to treatment with hybrids **1b–h**.

for this test was the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*pgc-1*). It is a promiscuous regulator of many genes involved in metabolism of lipids in the mitochondria.[2,37] Up-regulation of *pgc-1* is reported to enhance lipid oxidation.[2,37]

Assay results of the hybrids on *pgc-1* showed that all except **1d** and **1h** stimulated higher expression than the control (Fig. 4) with **1e**,**1f** and **1g** even stimulating more than insulin. As shown from the results, **1c** and **1g** exhibited the desired effects on the 3 genes consistently while **1d** was found to be inconsistent with regards to *pgc-1*.

The higher stimulating effect of **1g** compared to **1c** coupled with the relatively better potency of **1c** to **1d**, suggests that the presence of a non-polar short branched chain of the amine moiety might be important in the up-regulation of oxidative (*cpt-1* and *pgc-1*) and down-regulation of lipid accumulating (*acc-1*) genes.

In conclusion, a series of thiosemicarbazone-triazole hybrids were evaluated for their effect on *acc-1*, *cpt-1* and *pgc-1*. The results showed that **1g** followed by **1c** had the desired influence on the mitochondrial lipid metabolizing genes, *cpt-1*, *acc-1* and *pgc-1*. We are currently synthesizing analogues of **1g** for structural activity relationship studies and comparison with metformin in order to obtain a more potent potential agent as well as to study the mode of action of the respective agents.

Acknowledgments

We would like to acknowledge the University of Johannesburg for financial support and Professor N.J. Crowther from the University of the Witwatersrand for supplying cell cultures.

References and notes

1. Gao, Z.; Zhang, X.; Zuberi, A.; Hwang, D.; Quon, M. J.; Lefevre, M.; Ye, J. *Mol. Endocrinol.* **2004**, *18*, 2024.
2. Koves, T. R.; Li, P.; An, J.; Akimoto, T.; Slentz, D.; Ilkayeva, O.; Dohm, G. L.; Yan, Z.; Newgard, C. B.; Muoio, D. M. *J. Biol. Chem.* **2005**, *280*, 33588.
3. Bruce, C. R.; Thrush, A. B.; Mertz, V. A.; Bezaire, V.; Chabowski, A.; Heigenhauser, G. J. F.; Dyck, D. J. *Am. J. Physiol: Endocrinol. Metab.* **2006**, *291*, E99.
4. Dobbins, R. L.; Szczepaniak, L. S.; Bentley, B.; Esser, V.; Myhill, J.; McGarry, J. D. *Diabetes* **2001**, *50*, 123.
5. Patti, M. E.; Butte, A. J.; Crunkhorn, S.; Cusi, K.; Berria, R.; Kashyap, S.; Miyazaki, Y.; Kohane, I.; Costello, M.; Saccone, R.; Landaker, E. J.; Goldfine, A. B.; Mun, E.; Defronzo, R.; Finlayson, J.; Kahn, C. R.; Mandarino, L. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 8466.
6. Petersen, K. F.; Dufour, S.; Befroy, D.; Garcia, R.; Shulman, G. I. *New Engl. J. Med.* **2004**, *350*, 664.
7. Bruce, C. R.; Hoy, A. J.; Turner, N.; Watt, M. J.; Allen, T. L.; Carpenter, K.; Cooney, G. J.; Febbraio, M. A.; Kraegen, E. W. *Diabetes* **2009**, *58*, 550.
8. Vankoningsloo, S.; Piens, M.; Lecocq, C.; Gilson, A.; De Pauw, A.; Renard, P.; Demazy, C.; Houbion, A.; Raes, M.; Arnould, T. *J. Lipid Res.* **2005**, *46*, 1133.
9. Vankoningsloo, S.; De Pauw, A.; Houbion, A.; Tejerina, S.; Demazy, C.; De Longueville, F.; Bertholet, V.; Renard, P.; Remacle, J.; Holvoet, P.; Raes, M.; Arnould, T. *J. Cell Sci.* **2006**, *119*, 1266.
10. Hotamisligil, G. S.; Shargill, N. S.; Spiegelman, B. M. *Science* **1993**, *259*, 87.
11. Tejerina, S.; De Pauw, A.; Vankoningsloo, S.; Houbion, A.; Renard, P.; De Longueville, F.; Raes, M.; Arnould, T. *J. Cell Sci.* **2009**, *122*, 145.
12. Danforth, E., Jr *Nat. Genet.* **2000**, *26*, 13.
13. Kang, J.; Heart, E.; Sung, E. K. *Am. J. Physiol. Endocrinol. Metab.* **2001**, *280*, E428.
14. Rosen, E. D.; Spiegelman, B. M. *Nature* **2006**, *444*, 847.
15. Kusunoki, J.; Kanatani, A.; Moller, D. E. *Endocrine* **2006**, *29*, 91.
16. Paton, C. M.; Ntambi, J. M. *Am. J. Physiol. Endocrinol. Metab.* **2009**, *297*, E28.
17. Field, C.; Ryan, E.; Thomson, A.; Clandinin, M. *J. Biol. Chem.* **1990**, *265*, 11143.
18. Shillabeer, G.; Hornford, J.; Forden, J. M.; Wong, N. C. W.; Lau, D. C. W. *J. Lipid Res.* **1990**, *31*, 623.
19. Pedersen, O.; Kahn, C. R.; Flier, J. S.; Kahn, B. *Endocrinology* **1991**, *129*, 771.
20. Coleman, D. C. *Diabetologia* **1978**, *14*, 141.
21. Sung, J.-H.; Chon, J.-W.; Lee, M.-A.; Park, J.-K.; Woo, J.-T.; Kyoung, Y. *Nutr. Res. Pract.* **2011**, *5*, 503.
22. Morphy, R.; Kay, C.; Rankovic, Z. *Drug Discovery Today* **2004**, *9*, 641.
23. Shipman, C., Jr; Smith, H.; Drach, J. C.; Klayman, D. L. *Antiviral Res.* **1986**, *6*, 197.
24. Philip, V.; Suni, V.; Kurup, M. R. P.; Nethaji, M. *Polyhedron* **1931**, *2006*, 25.
25. Usman, A.; Razak, L. A.; Chantrapromma, S.; Fun, H. K.; Sreekanth, A.; Sivakumar, S.; Kurup, M. R. P. *Acta Cryst. C* **2002**, 461.
26. Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.
27. Zou, Y.; Zhao, Q.; Liao, J.; Hua, H.; Yu, S.; Chai, X.; Xu, M.; Wua, Q. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2959.
28. Livingstone, S. E. *Q. Rev. Chem. Soc.* **1965**, *19*, 386.
29. Modzelewska, B. B.; Jagiello, W. E. *Acta Pol. Pharm.* **2000**, *57*, 199.
30. Jin, J. Y.; Zhang, L. X.; Chen, X. X.; Zhang, A. J.; Zhang, H. L. *Molecules* **2007**, *12*, 297.
31. Oliva, C. G.; Jagerovic, N.; Goya, P.; Alkorta, I.; Elguero, J.; Cuberes, R.; Dordal, A. *ARKIVOC* **2010**, 127. and references cited there in.
32. Kinfe, H. H.; Belay, Y. H. S. *S. Afr. J. Chem.* **2013**, *66*, 130.
33. González-Barroso, M. M.; Anedda, A.; Gallardo-Vara, E.; Redondo-Horcajo, M.; Rodríguez-Sánchez, L.; Rial, E. *Biochim. Biophys. Acta* **2012**, *1817*, 1768.
34. Mahmood, K.; Naeem, M.; Rahimnajjad, N. A. *Eur. J. Intern. Med.* **2013**, *24*, 20.
35. Scheen, A. J.; Paquot, N. http://dx.doi.org/10.1016/j.diabet.2013.02.006.
36. *Materials and methods:* 3T3-L1 pre adipocytes cell lines were used in this study. The cells were cultured on 25 cm tissue culture plates containing Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% FCS and 1% penicillin/streptomycin/fungizone at 37 °C with 5% CO_2 and 95% humidity. For differentiation, cells were cultured with DMEM supplemented with 2% Horse serum and 1% penicillin/streptomycin/fungizone. For treatment of compounds, 5 μL of (10 mg/mL; dose–response studies were done using hybrid **1g** as a model and it was found that 10 mg/mL was optimal. Since the hybrids did not have significant differences in molar mass, 10 mg/1 mL were used in this study) compounds **1a–h** and 100 nM insulin were added to the differentiated cells together with 10 mL of DMEM and control was not treated. After 4 days treatment, the medium were changed with non-supplemented DMEM medium. After 4 h incubation, total RNA were extracted from the cells. Total RNA were isolated and purified from 3T3-L1 adipocytes using QIAzol lysis reagent (QIAGEN Sciences, USA) and RNA clean and Concentrator-25 (Inqaba Biotec, South Africa). Double stranded cDNA was synthesized from approximately 3 μg of total RNA using SuperScript III Reverse Transcriptase (Invitrogen, USA). Real time PCR were set up using SensiMix SYBER No-ROX kit (Bioline, UK) and were cycled according to the SensiMix kit instructions in Real time PCR system (Rotor-Gene 3000, QIAGEN). RNA concentrations were estimated by measuring the absorbance at 260 nm, and purity was assessed by the 260 nm/280 nm absorbance ratio. The integrity of RNA was validated by running samples on a 1% agarose gel. The total RNA were added to the following: Oligo dt, Random hexamer and dNTP. The RNA was then denatured at 65 °C for 5 min and reverse transcribed by adding 0.1 DTT, SuperScript III Reverse Transcriptase and first strand buffer followed by incubation at 25 °C for 5 min and 55 °C for 60 min. The reaction were inactivated by heated at 70 °C for 15 min. Real time PCR were performed in triplicate using Rotor-Gene 3000 PCR machine using SensiMix SYBER No-ROX kit and primers were used to amplify the region of following genes Acc-1, Cpt-1, and Pgc-1. The amplification occurred in a three step cycle: Denaturation at 95 °C for 5 s, Annealing at 60 °C for 10 s and extension at 72 °C for 15 s. Relative mRNA

expression was normalized to actin reference gene and calculated according to relative standard method. The primers used for qPCR are as follows: (1). Acc-1 Forward-TGC CTA TGA ACT CAA CAG CG; Acc-1 Reverse-ACA TTC TGT TTAGCG TGG GG; Cpt-1 Forward-CCA GGC TAC AGT GGG ACA TT; Cpt-1 Reverse-GAA CTT GCC CAT GTC CTT GT; Pgc-1 Forward-CAT TTG ATG CAC TGA CAG ATG GA; Pgc-1 Reverse-CCG TCA GGC ATG GAG GAA; Actin Forward-GAG ACC TTC AAC ACC CCA GCC; Actin Reverse-GGAGAGCATAGCCCTCGTAG.

37. Semple, R. K.; Crowley, V. C.; Sewter, C. P.; Laudes, M.; Christodoulides, C.; Considine, R. V.; Vidal-Puig, A.; O'Rahilly, S. *Int. J. Obesity* **2004**, *28*, 176.