Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1189–1192

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Basal SAR of a Novel Insulin Receptor Activator

Harold B. Wood, Jr.,[a,*] Regina Black,[a] Gino Salituro,[c] Deborah Szalkowski,[b] Zhihua Li,[b] Yan Zhang,[c] David E. Moller,[b] Bei Zhang[b] and A. Brian Jones[a]

[a]*Basic Chemistry, Merck Research Laboratories, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*
[b]*Molecular Endocrinology, Merck Research Laboratories, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*
[c]*Natural Product Drug Discovery, Merck Research Laboratories, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*

Received 17 February 2000; accepted 20 March 2000

Abstract—The synthesis and SAR of analogues prepared from novel insulin receptor activator **1** are described. Changes to the dihydroxyquinone core were not tolerated while functionalization of the two indoles contained in **1** resulted in little effect upon activation of the insulin receptor.

Many strategies for the treatment of diabetes have been devised,[1–4] however a small molecule, orally active, insulin receptor (IR) activator, that mimics the function of insulin, remains a logical but unrealized aspiration. Recently, Zhang and co-workers identified a compound (**1**) that is capable of both independently activating the IR in vitro and sensitizing it to exogenous insulin in vitro.[5,6] Furthermore compound **1** was capable of producing glucose lowering in traditional rodent models of diabetes after oral administration.[5] These tantalizing results demanded exploration of the ultimate potential of such compounds for the treatment of diabetes and the nature of their action. Determining what structural features of **1** contributed to its unique biological activity represented a necessary first step in such an enterprise.

As the natural product was readily available we initially focused on extracting as much basal information as possible directly from this material and it is these studies we now report. The structure–activity relationship (SAR) was pursued in three stages: (1) modifications of the quinone core, (2) selective functionalization of the indole nitrogens, and (3) alteration of the prenyl substituents.

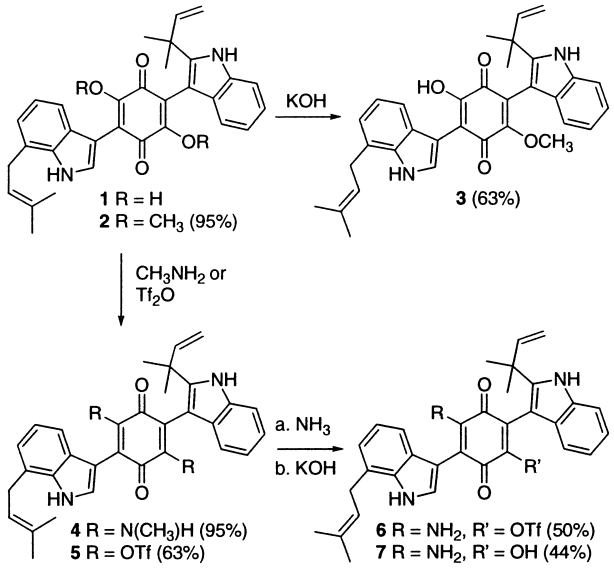
Conservative modifications to the quinone core were made as follows. Protection of the hydroxyl groups on the quinone was achieved with diazomethane to afford 2,4-dimethoxyquinone **2**. Upon mild treatment of **2** with base (1.1 equiv KOH, THF/water), a selective hydrolysis of one methoxy group was achieved to afford **3** after HPLC purification. While the regioselectivity of this reaction could never be conclusively confirmed, loss of the methoxy group proximal to the 7-prenyl indole was rationalized based upon the similar selectivity seen during BOC protection at the less sterically hindered 7-prenyl indole nitrogen (Scheme 2). Substitution of the methoxy for methyl amino groups[7] was accomplished to give **4**, when **2** was stirred with methyl amine in methanol at room temperature. Alternatively, reaction of **1** with triflic anhydride and 2,6-di-*t*-butyl-4-methyl pyridine gave bistriflate **5**. Treatment of **5** with one equivalent of ammonia in methanol resulted in a selective substitution yielding **6**. Refluxing **6** in KOH gave amino hydroxy quinone **7** in modest yield after HPLC purification (Scheme 1).

The ability of the quinone modified analogues to activate the IR were evaluated and compared to that of lead structure **1**.[8] As shown in Table 1, there appears to be a strict requirement for both the 2- and 4-hydroxy groups to maintain good levels of activation of the insulin receptor.

Turning attention to the indole heteroatom, a selective, rapid *N*-methylation occurred by phase-transfer alkylation[9] of **2** with 1 equiv of methyl iodide giving monomethyl indole **8** after KOH removal of the methoxy groups. With excess methyl iodide phase transfer alkylation was successful after 48 h to afford the *N*,*N*′-dimethyl indole **9** after hydrolysis of the methoxy

*Corresponding author. Tel.: +1-732–594-7592; fax: +1-732–594-9556; e-mail: blair_wood@merck.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00206-7



Scheme 1.

groups. Reaction of **2** with BOC anhydride and potassium carbonate in methylene chloride afforded a single compound in excellent yield after column chromatography. The proton NMR and mass spectrum confirmed that only one BOC group was present. The structure was assigned (vide infra) as that shown in compound **10**. The selectivity was rationalized on the basis that the 2-reverse prenyl indole was much more sterically hindered than the 7-prenyl indole. In fact, even under forcing conditions a second BOC group could not be introduced onto the second indole. Reaction of **10** with excess methyl iodide under PT alkylation conditions for extended reaction times gave the more hindered monomethyl quinone **11**[10] after exhaustive hydrolysis with KOH and HPLC purification (Scheme 2).

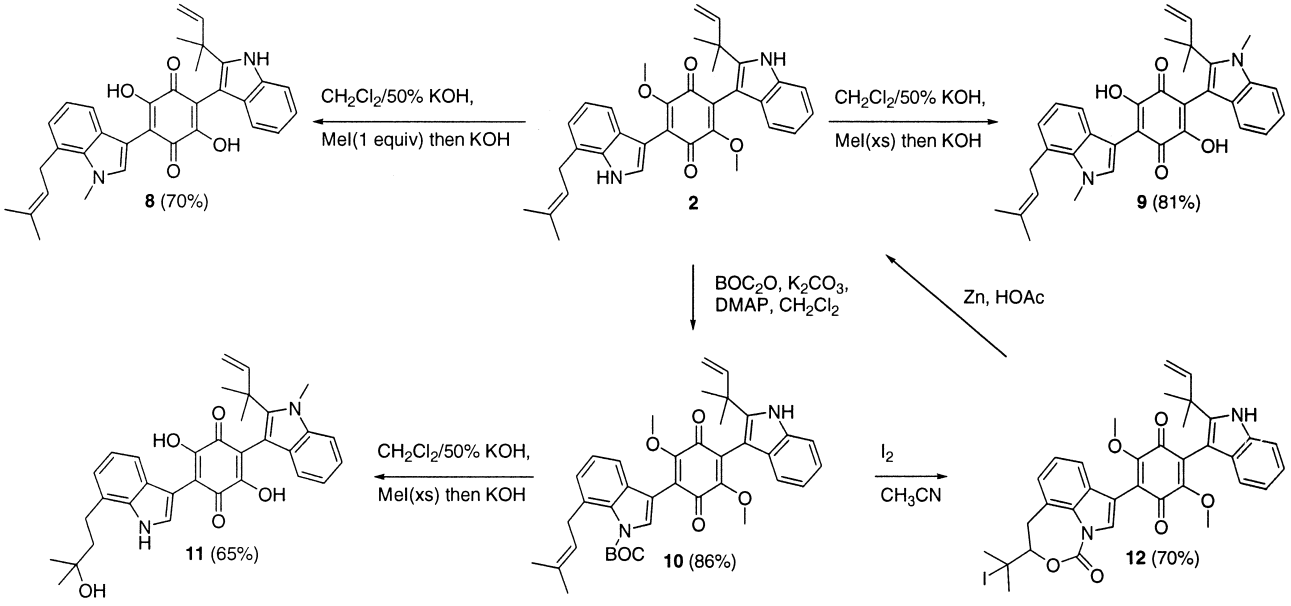
Table 1. % Activation of IR by quinone modified analogues **2**–**7** as compared to lead compound **1**

Compd	% Activation at 10 μM	Relative activity (X/1)
1	75	1.00
2	0	0
3	16	0.21
4	1	0.01
5	0	0
6	0	0
7	2	0.03

The regioselectivity of the introduction of the BOC group could not readily be determined using spectroscopic methods. However, reaction of **10** with iodine in anhydrous acetonitrile resulted in ring closure to cyclic iodo carbamate **12**. This chemical transformation allowed an unambiguous assignment of the regioselectivity in the formation of **10**. Reaction of **12** with Zn in HOAc then air oxidation returned intact **2** (Scheme 2).

Comparison of the activation of the IR by the *N*-monomethylated analogues indicate only a modest effect relative to lead compound **1**. The extremely low activation by *N*,*N*′-dimethyl analogue **9** is likely a consequence of the poor solubility of the compound in the assay media (Table 2).

Selective modification of the prenyl groups was also investigated. A carefully monitored catalytic hydrogenation of **1** with 10% Pd/C in ethyl acetate afforded a 2:1 mixture of **13** and **14** the di- and tetrahydro analogues, respectively, after re-oxidation of the hydroquinone to the quinone in air and HPLC purification. Interestingly, hydrogenation was the only reaction in this study that resulted in a selective reaction with the reverse prenyl



Scheme 2.

group. Alternatively, treatment of **1** with trifluoroacetic acid (TFA) in methylene chloride at room temperature gave quantitative yield of tertiary trifluoroacetate **15**, which in turn could be converted into alcohol **16** upon treatment with potassium carbonate in methanol then acidification (Scheme 3).

A wide range of reactions with the goal of selective olefin functionalization was attempted on **2** with little success. Typically, very complex reaction mixtures were obtained due to reactivity of the quinone core and workable reaction products could not be isolated from the crude reactions. In contrast to a simple dihydroxylation with osmium tetroxide which gave complex reaction mixtures, a selective dihydroxylation of the 7-prenyl group was achieved with the Sharpless asymmetric dihydroxylation system[11] to give high yield of diol **17** after removal of the methoxy groups with KOH and HPLC purification (Scheme 3).

Modifications to the prenyl groups had almost no effect upon the activation of the IR. Even when more sterically demanding functionality (trifluoroacetate **15**) or polar functional groups were added (monohydroxy **16** and dihydroxy **17**) the change in activation was negligible (Table 3).

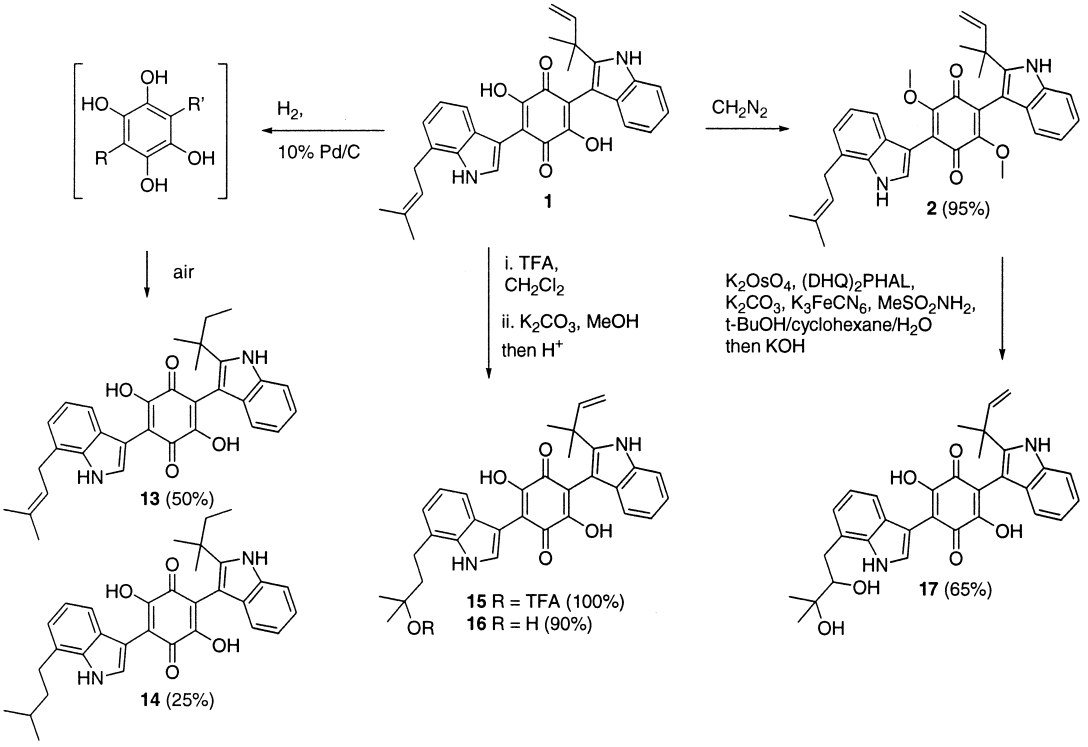
In summary, the direct investigation of the SAR of **1**, from **1**, suggests that the dihydroxyquinone core is required to maintain insulin receptor activation in this series of compounds but that the activity is insensitive to modifications of the flanking prenyl indoles.[12] Since quinones are known to participate in redox chemistry and to act as electrophiles, we have studied the role of the quinone moiety and searched for a replacement for this structural feature in these compounds. Given the apparent lack of importance of the prenyl groups to IR activation along with the limited ability to comprehensively evaluate the SAR of **1** due to its chemical reactivity, we resorted to de novo syntheses of substituted dihydroxyquinones[13] to more fully explore the SAR of this interesting new class of small molecule insulin sensitizers. These studies will be reported in due course.

Table 2. % Activation of IR by quinone modified analogues **8**, **9** and **11** as compared to lead compound **1**

Compd	% Activation at 10 μM	Relative activity (X/1)
1	75	1.00
8	24	0.32
9	3	0.03
11	79	1.05

Table 3. % Activation of IR by quinone modified analogues **13**–**17** as compared to lead compound **1**

Compd	% Activation at 10 μM	Relative Activity (X/1)
1	75	1.00
13	82	1.09
14	57	0.76
15	48	0.64
16	35	0.47
17	75	1.00



Scheme 3.

References and Notes

1. Hulin, B.; McCarthy, P. A.; Gibbs, E. M. *Curr. Pharmaceut. Des.* **1996**, *2*, 85.
2. Stevenson, R. W.; Gibbs, E. M.; Kreutter, D. K.; McPherson, R. K.; Clark, C. A.; Hulin, B.; Goldstein, S. W.; Parker, J. C.; Swick, A. G.; Treadway, J. L.; Hargrove, D. M.; Schulman, G. I. In *Diabetes Annual*; Marshall, S.; Home, P.; Rizza, R., Eds.; Elsevier Science: Amsterdam, 1995; Vol. 9, p 175.
3. Williams, G. *Lancet*. **1994**, *343*, 95.
4. Hulin, B. *Prog. Med. Chem.* **1994**, *31*, 1.
5. Zhang, B.; Salituro, G.; Szalkowski, D.; Li, Z.; Zhang, Y.; Royo, I.; Vilella, D.; Diez, M. T.; Pelaez, F.; Ruby, C.; Kendall, R. L.; Mao, X.; Griffin, P.; Calaycay, J.; Zierath, J. R.; Heck, J. V.; Smith, R. G.; Moller, D. E. *Science* **1999**, *284*, 974.
6. Salituro, G.; Zhang, Y.; Royo, I.; Vilella, D.; Pelaez, F.; Diez, M. T.; Ruby, C. Department of Natural Product Drug Discovery, Merck Research Laboratories, **1** was isolated from liquid cultures of a strain of the fungus *Pseudomassaria* sp. (American Type Culture Collection 74411), which was recovered from leaves of an undetermined plant collected near Kinshasa, Democratic Republic of Congo. For the production of the compound, the strain was grown in a medium containing (per l) D-mannitol (100 g), NZ-Amine (type E) (Quest International, Norwich, NY) (33 g), Difco-Yeast Extract (Becton Dickinson, Franklin Lakes, NJ) (10 g), ammonium sulfate (5 g), K_2HPO_4 (9 g). An organic extract of the broth was purified by gel filtration and two subsequent cycles of reverse phase HPLC. 1 crystallized as dark purple needles from ethyl acetate/hexane when stored at 4 °C.
7. Osman, A.-M. *J. Am. Chem. Soc.* **1957**, *79*, 966.
8. Assay Protocol: CHO.IR cells were cultured in 96-well plates (150,000 cells per well) for 24 h then serum-starved for 2 h before treatment with test compounds or insulin in the presence of 0.1% dimethyl sulfoxide (DMSO) in the medium for 20 min at 37 °C. Preparation of cell lysates, immunopurification of IR, and measurements of IRTK were performed as described in; Zhang, B.; Szalkowski, D.; Diaz, E.; Hayes, N.; Smith, R.; Berger, J. *J. Biol. Chem.* **1994**, *269*, 25735. Receptors were captured with antibody to IR (Ab-3, Oncogene Science Diagnostics, Cambridge, MA), and IRTK activity was measured with [γ-^{32}P]ATP and poly(Glu:tyr) (4:1) as substrate. The activities of test compounds were expressed as percentage of the maximal activity achieved with 100 nM insulin.
9. Luo, Y.-L.; Chou, T.-C.; Cheng, C. C. *J. Heterocycl. Chem.* **1996**, *33*, 113.
10. The tertiary alcohol in compound **11** is the result of addition of trifluoroacetic acid to the trisubstituted olefin during concentration of the fractions after HPLC purification. The hydroxy group has a negligible effect upon biological activity. See compound **16**, Table 3 for comparison.
11. Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, J.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768. The enantioselectivity of the dihydroxylation to yield diol **17** was not determined.
12. All compounds gave satisfactory analytical data.
13. Liu, K.; Wood, H. B.; Jones, A. B. *Tetrahedron Lett.* **1999**, *40*, 5119.