

Conjugates of Degraded and Oxidized Hydroxyethyl Starch and Sulfonylureas: Synthesis, Characterization, and in Vivo Antidiabetic Activity

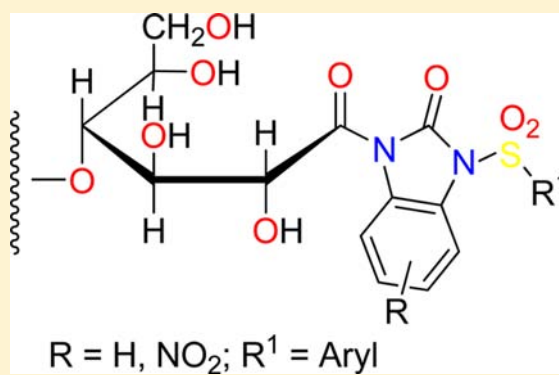
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S Supporting Information

ABSTRACT: Orally administered drugs usually face the problem of low water solubility, low permeability, and less retention in blood-stream leading to unsatisfactory pharmacokinetic profile of drugs. Polymer conjugation has attracted increasing interest in the pharmaceutical industry for delivering such low molecular weight (M_w) drugs as well as some complex compounds. In the present work, degraded and oxidized hydroxyethyl starch (HES), a highly biocompatible semisynthetic biopolymer, was used as a drug carrier to overcome the solubility and permeability problems. The HES was coupled with synthesized *N*-arylsulfonylbenzimidazolones, a class of sulfonylurea derivatives, by creating an amide linkage between the two species. The coupled products were characterized using GPC, FT-IR, ¹H NMR, and ¹³C NMR spectroscopy. The experiments established the viability of covalent coupling between the biopolymer and *N*-arylsulfonylbenzimidazolones. The coupled products were screened for their in vivo antidiabetic potential on male albino rats. The coupling of sulfonylurea derivatives with HES resulted in a marked increase of the hypoglycemic activity of all the compounds. 2,3-Dihydro-3-(4-nitrobenzensulfonyl)-2-oxo-1*H*-benzimidazole coupled to HES₁₀₁₀₀ was found most potent with a 67% reduction in blood glucose level of the rats as compared to 41% reduction produced by tolbutamide and 38% by metformin.



INTRODUCTION

The problem of solubility for orally administered drugs is a major challenge for formulation scientists. Reasonable solubility of the drugs is necessary to have good bioavailability through complete absorbance. Different technological approaches have been tried to solve the problem during pharmaceutical product development. The concept of using biopolymers as carrier, and to increase the water solubility of synthesized drugs, is definitely a right and proper way to improve the pharmacokinetics and bioavailability of such compounds. As a number of drugs have low water solubility, the concept of drug–polymer conjugation, and solubility properties of such systems, is prone to become more important in the future. As a result of polymer conjugation, the water solubility and stability of newly synthesized drugs, in general, increases along with the drug volume. The increased drug volume may help protect the drug from enzymatic and hydrolytic degradation. The polymer may also direct the drug polymer conjugate to specific sites in the body.¹

There are a large number of low-molecular-weight substances of commercial interest, especially active pharmaceutical ingredients, with limited or even prevented use because of low solubility in aqueous medium and short residence time.

The use of less soluble medicaments is also often associated with toxic side effects because of their deposition in organs such as liver and/or kidney.²

An approach followed in recent times for tackling these problems consists of coupling such less soluble substances to readily soluble biocompatible polymers. It is possible through coupling, on one hand, to increase the molecular weight above the renal threshold so that the plasma residence time of small molecules may be drastically increased, and on the other hand, the solubility in aqueous medium may be improved by the hydrophilic polymer portion.³ Most modifications to date have been carried out with polyethylene glycol (PEG) or dextran, PEG being generally preferred because it yields simpler products.^{2,3} Dextran conjugates often show high allergenicity, low metabolic stability, and in many cases low yields of the coupling reactions.⁴ Because of the polyfunctional nature of the molecule, the coupling usually yields a complex and heterogeneous mixture of conjugates creating complications and in some cases even death of the patient.⁵ There have

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likewise been reports of unpleasant or hazardous side effects, such as pruritus, hypersensitivity, and pancreatitis, on the use of PEG conjugates.⁶ In addition, the biological activity of the active ingredients is greatly reduced in some cases after PEG coupling.⁷ Moreover, the metabolism of degradation products of PEG conjugates is still substantially unknown and possibly represents health risks.⁴

There is still a need for physiologically well tolerated alternatives to dextran or PEG conjugates to improve the solubility of poorly soluble low-molecular-weight substances. The biopolymer hydroxyethyl starch (HES) is a good choice for such coupling reactions.⁸ A substantial advantage of HES is that it has already been approved by the authorities as a biocompatible plasma expander and is employed clinically on large scale.⁴

To be used as drug carrier, the HES must have a certain size of the molecule (or M_w) to be eliminated through the kidney. The large molecules of HES may not be eliminated through the kidney and accumulate causing serious problems. Therefore, HES needs to be degraded to lower M_w fractions to meet the removal requirements. The pharmacokinetic behavior of medium and low M_w starches has been investigated by many researchers. It has been observed that excellent degradability of low M_w HES has not only no quantifiable reduction in clinical efficacy,^{9,10} but also improved safety profile due to more rapid elimination.¹¹ The pharmacokinetic studies have confirmed that low M_w starch is safe not only in healthy human subjects,¹² but also in patients with mild to severe renal impairment.¹³

In addition, HES needs to be chemically modified to generate suitable functional groups for coupling with the drug. For this purpose, the successful selective degradation and oxidation of HES to generate carboxylic acid group at the chain ends has recently been reported from this laboratory.¹⁴

Most of the marketed antidiabetic drugs are the sulfonyl derivatives of urea. Benzimidazolones (1,3-dihydro-2H-benzimidazol-2-one) are a unique class of cyclic urea derivatives and have shown remarkable activity as hypoglycemic agents.¹⁵ In addition, a wide variety of other biochemical and pharmacological properties of these compounds have also been demonstrated. They antagonize neurotransmitters,¹⁶ inhibit aldose reductase,¹⁷ show antiulcer and antisecretory properties,¹⁸ enhance pulmonary surfactant secretion, and modulate ion channels.¹⁹ Several of these compounds show activity against leukemia.²⁰ The conversion of these cyclic urea derivatives to their sulfonyl derivatives may result in a molecule having enhanced antidiabetic activity. In addition, the benzimidazolone core may act as aldose reductase inhibitor, and hence help to prevent cataract formation—a complication associated with diabetes. The coupling of these sulfonyl cyclic urea derivatives with HES is supposed to increase their water solubility, and hence, the concentration of the drug outside the cell that may result in higher penetration into the cell. Second, hydroxyethylation of starch reduces its *in vivo* degradation rate.²¹ Unsubstituted anhydroglucose units are more prone to enzymatic degradation by α -amylase. Thus, hydroxyethylation may restrict the rate of enzymatic degradation of the HES molecule and prolong intravascular retention time. The polymer may also direct the drug–polymer conjugate to specific sites in the body.¹ The amide linkage created by the coupling of sulfonyl cyclic urea derivatives with HES may undergo *in vivo* hydrolysis to release the drug molecule.

In the present paper, we report the synthesis of a number of *N*-arylsulfonyl derivatives of benzimidazolones, their coupling

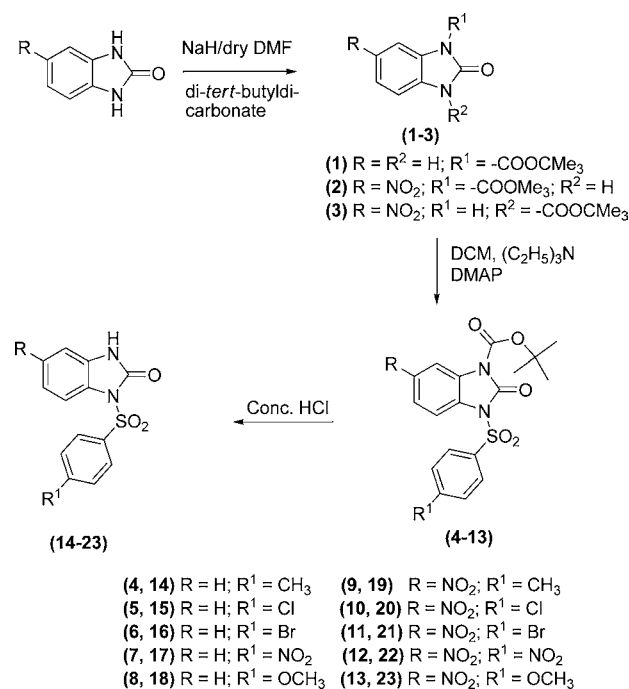
with HES, and antidiabetic activity of the resultant conjugates, in line with our previous studies.^{22–24} The activity of the neat molecules was also determined and is being compared with that of the conjugates.

RESULTS AND DISCUSSION

The synthesis of the drug–HES conjugates was accomplished in three steps. The first step involved the preparation of antidiabetic drugs from benzimidazolones. Second, coupling of the drug and the polymer was carried out through an amide linkage. Finally, the synthesized drugs and their coupled products were screened for their *in vivo* antidiabetic activity.

Synthesis of *N*-Arylsulfonylbenzimidazolones (14–23). A particular problem observed in the preparation of benzimidazolone derivatives is the selective functionalization of a single ureido nitrogen atom. The direct monoalkylation of one of the nitrogen atoms of benzimidazolone is not feasible,^{25,26} particularly with unsymmetrical benzimidazolones. However, a single alkoxycarbonyl moiety could be easily and selectively introduced directly to these cyclic urea derivatives.²⁷ An alkoxycarbonyl group, such as a *tert*-butoxycarbonyl group, is an attractive and potentially useful protecting group for this purpose, since it effectively combines reasonable chemical stability with ready removal under either mild alkaline²⁸ or acidic conditions.²⁹ The protecting group facilitates functionalization of the other degenerate nitrogen atom of the heterocyclic ring under mild conditions (Scheme 1).

Scheme 1. Synthesis of *N*-Arylsulfonylbenzimidazolones (14–23)



2(3H)-Benzimidazolones were synthesized by the reaction of *o*-phenylenediamine with urea in the presence of acetic acid as catalyst.³⁰ The selective protection of a single nitrogen atom of benzimidazolones was successfully achieved in high yield under inert conditions of argon in dry DMF using di-*tert*-butyldicarbonate. In the case of 5-nitro-2(3H)-benzimidazolone as starting material, two isomers were obtained, which were separated by flash column chromatography using silica gel as an

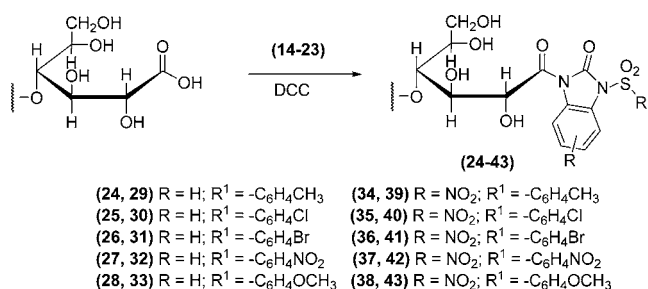
adsorbent and ethyl acetate:*n*-hexane (1:2) as eluent. The major regioisomer (**2**) was used for further derivatization. The synthesis of *mono*-protected benzimidazolones (**1–3**) was indicated in the IR spectra by the appearance of two strong carbonyl absorptions: one for amide and the other for ester linkage. The confirmation of synthesis was witnessed in the ^1H NMR spectra by the presence of only one downfield signal for NH proton in the range 11.50–9.75 ppm. A singlet in the range of 1.59–1.36 ppm confirmed the presence of nine protons of the *t*-butyl group. In ^{13}C NMR spectra, the ester carbonyl carbons resonated in the range of 152.0–148.0 ppm. The formation of the products was further confirmed by EIMS analysis where a low intensity molecular ion peak was observed at $m/z = 234$ for compound **1** and at $m/z = 279$ for compound **2**. The base peak in both compounds appeared due to the loss of *tert*-butoxycarbonyl group. The loss of CO_2 molecule from the molecular ion was also observed.

The sulfonation of *mono*-protected benzimidazolones (**1** and **2**) with arylsulfonyl chlorides was carried out in dry dichloromethane using triethylamine as a base and dimethylaminopyridine as a catalyst. In FT-IR spectra of the synthesized sulfonylated benzimidazolones (**4–13**), a band in the region 1198–1190 cm^{-1} for asymmetric and a second band in the range of 1360–1345 cm^{-1} for symmetric stretching vibrations of the sulfonyl group was observed. The confirmation of synthesis was achieved in ^1H NMR spectra by the appearance of additional signals in the aromatic region and absence of $-\text{NH}$ proton signals observed in the starting materials. Additional signals in the aromatic region were also observed in ^{13}C NMR spectra of these compounds. The structures of sulfonylated products (**4–13**) were further confirmed by EIMS analysis. The mass spectra exhibited a molecular ion peak with low intensity for all the compounds. The base peak resulted by the loss of $-\text{COOCMe}_3$ fragment from the molecular ion. The loss of SO_2 molecule from the molecular ion was also observed.

The *mono*-protected sulfonylated benzimidazolones (**4–13**) were successfully deprotected on treatment with concentrated hydrochloric acid in diethyl ether at room temperature to give *N*-arylsulfonylbenzimidazolones (**14–23**). The formation of *N*-arylsulfonylbenzimidazolones (**14–23**) was witnessed by the re-emergence of the $-\text{NH}$ band in the region of 3365–3375 cm^{-1} in the IR spectra. The formation of products was confirmed in ^1H NMR spectra by the reappearance of downfield signal for the $-\text{NH}$ proton in the range 9.98–9.20 ppm. The absence of a singlet at around 1.6 ppm for nine protons of the *t*-butyl group further confirmed the formation of products. In ^{13}C NMR spectra, the signal for only one carbonyl carbon in the range of 150.1–165.5 ppm also confirmed the synthesis. The formation of *N*-arylsulfonylbenzimidazolones (**14–23**) was further confirmed by EIMS analysis. The molecular ion peak was observed in all cases. The loss of the Ar-SO_2 group resulted in base peaks. The loss of SO_2 from the molecular ion also gave a prominent fragment.

Coupling of *N*-Arylsulfonylbenzimidazolones with HES. The carboxylic acid group in oxidized HES and the free N-H in the arylsulfonylbenzimidazolones were made to react for coupling of the drug with HES. The oxidized HES of M_w 17 500 and 10 100 g/mol were used for coupling purposes to afford the products **24–33** and **34–43**, respectively. The reaction was performed in the presence of dicyclohexyl carbodiimide (DCC) as an activator in a mixture of DMF and water (Scheme 2). Two strategies were tried for the coupling reactions: the first using a two-step reaction with a

Scheme 2. Synthesis of Coupled Products (24–43**) from Oxidized HES₁₇₅₀₀ and HES₁₀₁₀₀**



preactivation of the polymeric acid to acid chloride, and the second using a direct approach without preliminary activation of oxidized HES. It was observed that there is no specific advantage in a two-step reaction in terms of yield of the reaction. Therefore, all the coupling reactions were carried out using the direct approach.

For structure elucidation of coupled products (**24–43**), GPC, FTIR, and NMR spectroscopic techniques were used. The increase in relative molecular weight of conjugates and change in their elution times, as measured by GPC, supported the formation of coupled products. The coupling of *N*-arylsulfonylbenzimidazolones with oxidized HES was also indicated in the IR spectra of the products by the presence of a band in the range 1670–1645 cm^{-1} attributed to the stretching vibrations of the $\text{C}=\text{O}$ functionality of the product. In nitro-group containing conjugates, the bands for NO_2 stretchings were observed in the spectral regions of 1585–1450 and 1420–1375 cm^{-1} . The ^1H NMR spectra confirmed the formation of coupled products (**24–43**) by the presence of additional signals for aromatic protons. The absence of the downfield signals for $-\text{NH}$ (of benzimidazolone nucleus) and carboxylic acid (of the oxidized HES) protons also provided the evidence for coupling. The methyl and methoxy protons of *N*-arylsulfonylbenzimidazolones were not assignable in most cases due to overlapping with HES signals. The signal for carbonyl carbon of the amide group appeared in the range of 165.1–175.5 ppm in ^{13}C NMR spectra of the coupled products (**24–43**).

Solubility of the Coupled Products. The solubility of conjugated products was determined and compared with that of the respective drugs. A comparison of the solubility of the conjugated products and the neat drugs reveals that the solubility of drugs increases many-fold after its coupling with HES (Table 1). When the drugs were coupled to oxidized HES₁₀₁₀₀, the maximum increase in solubility (566-fold) observed was that of the drug **18** followed by 540-fold for compound **17**. The minimum increase in the solubility observed was 61-fold for compound **21**.

In Vivo Hypoglycemic Activities. The in vivo hypoglycemic activities of *N*-arylsulfonylbenzimidazolones (**14–23**) and their conjugates with HES (**24–33**) were tested on male albino rats of the Dawley-Sprague family according to well established methods.²²

The in vivo hypoglycemic activities of *N*-arylsulfonylbenzimidazolones (**14–23**) were measured at two different concentrations, i.e., 20 and 40 mg per kg of the rats' body weight. It was observed that all the compounds were active, in general, at a dose of 20 mg of the test compound. However, the activity was more pronounced at a dose of 40 mg per kg of the rats' body weight. The activities of all the compounds at a dose

Table 1. Solubility of Drugs (14–23) and their coupled products with HES₁₀₁₀₀

drug	solubility ^a (mol × 10 ⁻⁴ /5 mL)	solubility of the drug bound to HES ₁₀₁₀₀ ^a (mol × 10 ⁻⁴ /5 mL)	increase in solubility (fold)
14	0.034	5.65	166
15	0.041	9.53	232
16	0.021	1.56	74
17	0.062	33.51	540
18	0.070	39.64	566
19	0.021	2.74	131
20	0.027	3.04	113
21	0.014	0.861	61
22	0.025	4.71	188
23	0.030	7.73	258

^aAverages are means of three determinations ± SD. Values are significantly different ($p < 0.05$).

of 40 mg demonstrated a remarkable decrease ($p < 0.0001$) in the blood glucose level and were comparable to both the standard drugs, glucophage and tolbutamide, used at a dose of 150 mg per kg of the rats' body weight (Figure 1). The most

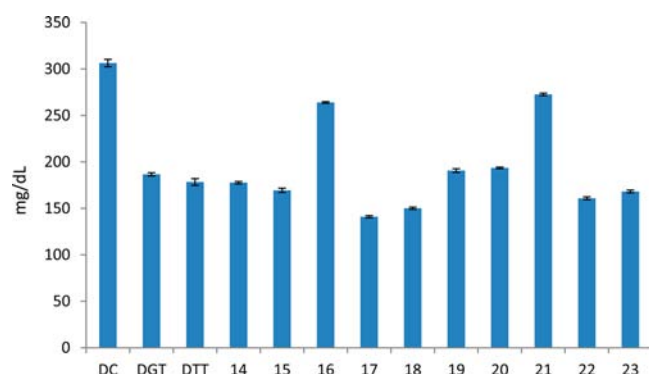


Figure 1. Comparison of hypoglycemic activity of the commercial drugs and compounds 14–23 in diabetic rats' model. $p < 0.0001$. Sample size: 5 male adult rats. Statistical Analysis: Tukey HSD test.

active compounds in the series were 17 and 18 with a reduction in the blood glucose level of 54% and 51%, respectively. These compounds have a nitro and methoxy substituent, respectively, on the phenylsulfonyl part of the products. However, it is interesting to note that when a nitro substituent is present on the benzene ring of the benzimidazolone nucleus, it has adverse effects on the hypoglycemic activity of the compounds as witnessed in compounds 19–23. The substituent effects in the two series of compounds (14–18 and 19–23) were found parallel to one another as can be observed from Table 2. Compounds 16 and 21 were the least active compounds in the series with a reduction in the blood glucose level of 14% and 12%, respectively. Both these compounds have a bromine atom as a substituent at the phenylsulfonyl part of the molecule.

The *N*-arylsulfonylbenzimidazolones coupled products (24–33) with HES₁₇₅₀₀ were screened for their hypoglycemic activity at the dose levels of 20 and 40 mg per kg of the rats' body weight. The activity of the compounds increased in general and all the coupled products (24–33) were found more active than the standard drugs (glucophage and tolbutamide) except compounds 26 and 31 (Table 3). However, the substituent effects were found parallel to those of the uncoupled *N*-arylsulfonylbenzimidazolones (14–23), the most active compounds being with a nitro (in compound 27) or a methoxy (in compound 28) group as substituents in the phenylsulfonyl part of the molecule. These compounds exhibited 57% and 58%

reduction in the blood glucose levels of the rats, respectively. The least active compounds (26 and 31) were with a bromine substituent in the arylsulfonyl part of the compounds. Again, the compounds substituted by a nitro group at position 5 of the nucleus were found less potent than their unsubstituted counterparts.

The *N*-arylsulfonylbenzimidazolones (14–23) were also coupled to HES₁₀₁₀₀ to study and compare hypoglycemic activities. On coupling to HES₁₀₁₀₀ the activities of the coupled products (34–43) were even higher ($p < 0.0001$) as depicted in Figure 2. This higher activity of the products (34–43)

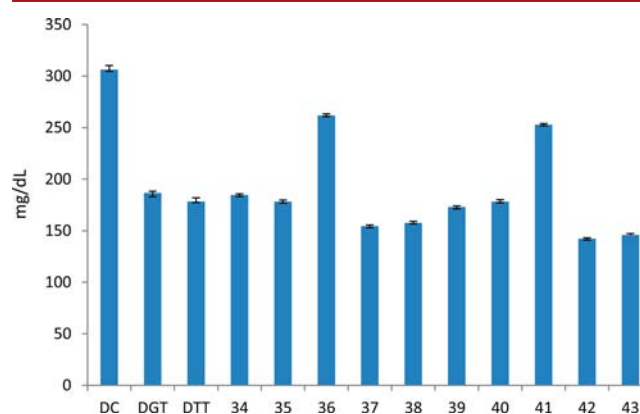


Figure 2. Comparison of hypoglycemic activity of the commercial drugs and compounds 34–43 in diabetic rats model. $p < 0.0001$. Sample size: 5 male adult rats. Statistical Analysis: Tukey HSD test.

coupled with HES₁₀₁₀₀ as compared to compounds (24–33) coupled with HES₁₇₅₀₀ may be attributed to the increased solubility of the product in water, hence higher availability of the drug. The most active products were 37 and 38, causing a reduction in the blood glucose of 67% and 65%, respectively (Table 4). There is a significant enhancement in the activity when compared to the values of 54% and 51% reduction observed for the uncoupled drugs 17 and 18. Although products 36 and 41 were least active of all these coupled products, their activity too was higher than that of the corresponding uncoupled products (16 and 21).

It may be observed from Tables 2–4 that the synthesized compounds (14–23) are reasonably active when compared to the standard commercial drugs (glucophage and tolbutamide) and the coupled products are even more potent than the neat compounds. This increased activity may be attributed to the increased solubility of the drugs on coupling with HES. The continuous increase in the activity with time indicates the

Table 2. Hypoglycemic Activities of *N*-Arylsulfonylbenzimidazolones (14–23)

compound	quantity (mg/kg)	blood glucose (mg/dL)			% reduction ^a (after 12 h)
		3 h	6 h	12 h	
NC (with vehicle)			98.34 ± 2.3		
DC	200		306.3 ± 8.6 (after 5 days)		
DGT	150	196.83 ± 2.98	194.71 ± 3.24	186.57 ± 3.32	38
DTT	150	188.30 ± 5.8	183.45 ± 5.62	178.32 ± 6.1	41
14	20	284.71 ± 2.3	269.54 ± 2.5	220.51 ± 2.3	28
	40	270.01 ± 2.8	245.02 ± 2.3	177.63 ± 1.76	43
15	20	269.51 ± 1.7	235.84 ± 2.9	209.45 ± 1.33	31
	40	245.02 ± 1.9	211.08 ± 1.10	169.40 ± 2.76	45
16	20	290.96 ± 2.1	279.48 ± 1.35	274.42 ± 1.26	10
	40	280.97 ± 2.33	266.48 ± 1.54	263.98 ± 1.1	14
17	20	257.25 ± 1.73	248.09 ± 1.12	199.08 ± 2.7	35
	40	251.12 ± 1.24	214.43 ± 1.89	140.89 ± 1.16	54
18	20	260.31 ± 1.5	251.12 ± 2.67	192.95 ± 1.32	37
	40	248.07 ± 2.4	220.51 ± 3.15	150.08 ± 1.74	51
19	20	290.57 ± 2.5	273.15 ± 1.2	234.26 ± 3.5	24
	40	282.10 ± 1.2	252.22 ± 0.9	190.65 ± 2.8	39
20	20	276.12 ± 2.1	250.28 ± 1.1	241.15 ± 1.5	22
	40	241.24 ± 2.6	210.48 ± 2.5	192.80 ± 2.6	36
21	20	299.28 ± 3.6	282.29 ± 2.2	281.90 ± 2.9	08
	40	289.64 ± 3.1	278.12 ± 2.9	272.80 ± 2.1	12
22	20	268.48 ± 2.8	255.28 ± 1.2	210.28 ± 1.5	32
	40	262.80 ± 2.1	251.10 ± 1.6	160.85 ± 2.7	47
23	20	272.42 ± 2.0	261.95 ± 2.5	211.34 ± 1.9	31
	40	258.10 ± 2.7	238.15 ± 3.2	168.34 ± 1.5	45

^a% change in blood glucose was calculated using formula %change = $[(T_c - T_t)/T_c] \times 100$, where T_c = values before administration of drug, T_t = values after administration of drug; data are expressed as mean ± SD; NC = normal control; DC = diabetic control; DGT = diabetic glucophage treated; DTT = diabetic tolbutamide treated.

increased retention of the drugs in bloodstream. This may be useful as a daily dose of the medicine. Furthermore, the compound treated rats were found normal and no toxic reaction or mortality was observed during this investigation.

CONCLUSIONS

In conclusion, two series of *N*-arylsulfonylbenzimidazolones were synthesized using a multistep reaction sequence. The synthesized *N*-arylsulfonylbenzimidazolones were successfully coupled with oxidized HES through an amide linkage. The in vivo antidiabetic activity of the synthesized compounds and their coupled products with HES was evaluated and compared with that of marketed drugs, tolbutamide and glucophage. All the synthesized compounds were found active in general, but their coupled products with HES were found even more active, as expected. 2,3-Dihydro-3-(4-nitrobenzenesulfonyl)-2-oxo-1*H*-benzimidazolone (17) was found most potent of all the synthesized compounds with a 54% reduction in blood glucose level of the rats as compared to 41% reduction produced by tolbutamide and 38% by glucophage. On coupling with HES₁₀₁₀₀, the activity increased further to 67% reduction in blood glucose level as compared to 54% by the drug itself. The higher activity of the drug may be attributed to its increased solubility affecting the availability of drug. However, it was observed that the compounds substituted with a nitro group in the aromatic ring of benzimidazolone nucleus were in general less potent than their unsubstituted counterparts. The compound treated rats were found normal and no toxic reaction or mortality was observed during this investigation.

MATERIALS AND METHODS

General. The reagents used were of high purity grade. Hydroxyethyl starch with molar mass of 70 000 g/mol was a kind gift from Serumwerk, Bernburg, Germany. The oxidizing agent sodium hypochlorite NaOCl (13%), sodium hydroxide (3% solution), α -amylase bacterial enzyme (source: *Bacillus licheniformis*, EC number 3.2.1.1), calcium chloride solution (2.5 mM/L Ca²⁺), *N,N*-dicyclohexylcarbodiimide (DCC), and *N,N*-dimethylaminopyridine (DMAP) were purchased from Sigma-Aldrich (Germany). Diethyl ether and dichloromethane were supplied by Lab-scan (Germany). Tetrahydrofuran (THF), triethylamine (TEA), benzoyl chloride, chloroform, carbon tetrachloride, and methanol were purchased from Riedel-deHaën (Germany). Acetone, ethyl acetate, and ethanol were obtained from commercial sources and distilled before use. All the reactions were monitored using precoated silicagel-60 HF₂₅₄ TLC plates and visualized under UV at 254 nm. Flash column chromatography was carried out on silica gel (35–70 μ m, Grace). Melting points of the compounds were determined in open capillaries using Gallenkamp melting point apparatus and are uncorrected. FT-IR spectra were recorded on a Hitachi model 270–50 spectrophotometer or on a Bruker Tensor 37 spectrophotometer as KBr discs; ¹H NMR spectra were recorded on a Bruker Avance 300 or 400 MHz Gemini 2000 (Varian) NMR spectrophotometer using TMS as an internal standard. The multiplicities are abbreviated as s = singlet, d = doublet, dd = doublet of doublet, ad = apparent doublet, t = triplet, at = apparent triplet, dt = doublet of triplet, td = triplet of doublet, q = quartet, and m = multiplet. The EIMS were recorded on a MAT-112-S machine at 70 eV. Determination and comparison study of M_w and M_n were carried out on

Table 3. Hypoglycemic Activities of the Coupled Products of *N*-Arylsulfonylbenzimidazolones with HES₁₇₅₀₀ (24–33)

compound	quantity (mg/kg)	blood glucose (mg/dL)			% reduction ^a (after 12 h)
		3 h	6 h	12 h	
NC (with vehicle)			98.34 ± 2.3		
DC	200		306.3 ± 8.6 (after 5 days)		
DGT	150	196.83 ± 2.98	194.71 ± 3.24	186.57 ± 3.32	38
DTT	150	188.30 ± 5.8	183.45 ± 5.62	178.32 ± 6.1	41
24	20	299.85 ± 2.4	288.45 ± 2.1	217.62 ± 1.35	29
	40	287.51 ± 2.5	269.10 ± 1.53	171.83 ± 1.5	45
25	20	290.45 ± 2.92	275.82 ± 1.42	199.65 ± 1.2	35
	40	278.02 ± 2.14	259.14 ± 2.63	158.51 ± 3.3	48
26	20	295.16 ± 2.43	284.45 ± 2.2	270.21 ± 1.52	11
	40	290.1 ± 3.26	282.68 ± 2.8	260.92 ± 1.53	15
27	20	272.3 ± 2.1	257.12 ± 1.6	192.28 ± 1.52	37
	40	268.4 ± 1.6	259.73 ± 1.2	132.81 ± 1.25	57
28	20	265.2 ± 2.8	254.32 ± 2.15	183.52 ± 2.4	41
	40	259.21 ± 2.4	242.32 ± 1.8	129.82 ± 1.24	58
29	20	288.90 ± 1.4	272.30 ± 2.7	228.20 ± 0.7	26
	40	281.78 ± 2.2	252.76 ± 2.1	184.62 ± 1.1	41
30	20	274.96 ± 2.8	233.18 ± 2.2	232.98 ± 2.7	25
	40	238.82 ± 2.1	204.86 ± 1.6	178.40 ± 1.9	42
31	20	296.38 ± 2.3	280.90 ± 1.2	279.98 ± 2.8	10
	40	287.75 ± 2.2	275.10 ± 1.6	261.82 ± 3.1	15
32	20	262.50 ± 2.5	250.65 ± 2.7	201.52 ± 1.2	35
	40	258.28 ± 1.8	239.40 ± 3.2	155.25 ± 1.1	51
33	20	267.90 ± 2.1	261.95 ± 3.6	204.20 ± 1.6	33
	40	256.45 ± 2.5	228.30 ± 1.7	157.94 ± 2.3	49

^a% change in blood glucose was calculated using formula %change = $[(T_c - T_t)/T_c] \times 100$, where T_c = values before administration of drug, T_t = values after administration of drug; data are expressed as mean ± SD; NC = normal control; DC = diabetic control; DGT = diabetic glucophage treated; DTT = diabetic tolbutamide treated.

Viscotek 270 dual detector GPC/SEC and Waters GPC equipped with a Knauer pump, two PSS columns, and RI detector (W410), using a solution of NaNO₃ in water (concentration 1–2%) as eluent. Polyethylene glycol calibration was used to calculate the molar masses. EBA 21 Hettich Zentrifuge was used with disposable centrifuge tubes for centrifugation.

General Method for the Synthesis of *tert*-Butyl 2,3-dihydro-2-oxobenzimidazole-1-carboxylates (1–3). To a solution of benzimidazolone (0.015 mol) in dry *N,N*-dimethylformamide (5.0 mL) was added sodium hydride (0.015 mol) under argon.²⁷ After stirring for 40 min, di-*tert*-butyldicarbonate (0.015 mol) was added dropwise from a dropping funnel and the mixture stirred for 24 h at 20 °C. The *N,N*-dimethylformamide was removed in vacuo, and the residue was treated with saturated ammonium chloride solution and extracted with ethyl acetate (3 × 50 mL). The solvent was removed in vacuo and the product purified by flash column chromatography using ethyl acetate:*n*-hexane (1:4) as eluent. In case of 5-nitro-2(3*H*)-benzimidazolone two isomers were obtained, which were separated by flash column chromatography using ethyl acetate:*n*-hexane (1:2) as eluent.

General Method for the Synthesis of 2,3-Dihydro-2-oxo-3-benzenesulfonyl-1*H*-benzimidazole-1-carboxylic acid, 1,1-Dimethylethyl esters (4–13). To the mono-protected benzimidazolone (0.01 mol) dissolved in dry dichloromethane (50.0 mL) was added triethylamine (0.01 mol) as a base and dimethylaminopyridine (1–2 crystals) as a catalyst. To this well-stirred solution, arylsulfonyl chloride (0.11 mol) was added portionwise and stirring continued at 20 °C for 3 h. The mixture was diluted with 1 N HCl (10 mL) and

extracted with dichloromethane (3 × 30 mL). The solvent was removed in vacuo and the crude product recrystallized from chloroform:*n*-hexane (1:3).

General Method for Synthesis of *N*-Arylsulfonylbenzimidazolones (14–23). In a 100 mL round-bottom flask, 1.3 mmol of monoprotected sulfonylated benzimidazolone was dissolved in acetonitrile (5.0 mL) and conc. hydrochloric acid (2.5 mL), separately dissolved in diethyl ether (6.0 mL) was added dropwise. The mixture was stirred at 20 °C for 3 h, concentrated on a rotary evaporator, and triturated with diethyl ether to give analytically pure monosulfonylated benzimidazolones.

General Method for Coupling of Arylsulfonylbenzimidazolones with Oxidized HES (24–43). Oxidized HES¹⁴ (HES₁₇₅₀₀ or HES₁₀₁₀₀; 0.00015 mol) was taken in 3.0 mL of DMF/distilled water (equal volumes) under magnetic stirring (5 rpm) at room temperature. After 30 min, DCC (0.002 mol) and DMAP (0.001 mol), dissolved separately in 5.0 mL water, were added slowly to the reaction mixture from a dropping funnel. After another 30 min, arylsulfonylbenzimidazolone (0.002 mol) dissolved in DMF/distilled water (3.0 mL), was added slowly under constant stirring. The reaction mixture was left for 24 h under moderate stirring. Thereafter, the solvent was evaporated under vacuum, the product taken in water, washed with ethyl acetate (2 × 5.0 mL), and precipitated using acetone. The product was filtered, washed with methanol, and air-dried. The solid product was dissolved in 50.0 mL of water and further purification was achieved by dialysis against distilled water for 48 h in a dialysis membrane. The solution was finally lyophilized, and the product was analyzed by GPC.

Table 4. Hypoglycemic Activities of the Coupled Products of *N*-Arylsulfonylbenzimidazolones with HES₁₀₁₀₀ (34–43)

compound	quantity (mg/kg)	blood glucose (mg/dL)			%reduction ^a (after 12 h)
		3 h	6 h	12 h	
NC (with vehicle)			98.34 ± 2.3		
DC	200		306.3 ± 8.6 (after 5 days)		
DGT	150	196.83 ± 2.98	194.71 ± 3.24	186.57 ± 3.32	38
DTT	150	188.30 ± 5.8	183.45 ± 5.62	178.32 ± 6.1	41
34	20	278.29 ± 2.2	266.47 ± 2.2	199.10 ± 1.23	35
	40	266.41 ± 2.92	251.09 ± 1.62	155.63 ± 1.9	49
35	20	266.51 ± 3.12	214.37 ± 1.76	168.65 ± 1.7	45
	40	238.9 ± 1.86	186.79 ± 2.45	149.40 ± 3.4	52
36	20	281.76 ± 2.56	269.48 ± 2.21	264.42 ± 1.62	15
	40	284.81 ± 3.42	266.45 ± 2.5	244.98 ± 1.72	20
37	20	251.17 ± 1.57	241.9 ± 1.76	159.08 ± 1.43	48
	40	248.08 ± 2.3	223.52 ± 1.35	101.13 ± 1.14	67
38	20	254.25 ± 2.6	248.12 ± 1.65	153.15 ± 1.62	50
	40	241.88 ± 2.1	186.83 ± 2.7	107.23 ± 1.84	65
39	20	288.90 ± 1.4	272.30 ± 2.7	221.20 ± 0.7	29
	40	281.78 ± 2.2	252.76 ± 2.1	173.62 ± 1.1	45
40	20	274.96 ± 2.8	233.18 ± 2.2	223.98 ± 2.7	28
	40	238.82 ± 2.1	204.86 ± 1.6	178.40 ± 1.9	43
41	20	296.38 ± 2.3	280.90 ± 1.2	277.98 ± 2.8	11
	40	287.75 ± 2.2	275.10 ± 1.6	252.82 ± 3.1	19
42	20	262.50 ± 2.5	250.65 ± 2.7	190.84 ± 1.2	38
	40	258.28 ± 1.8	239.40 ± 3.2	142.25 ± 1.1	54
43	20	267.90 ± 2.1	261.95 ± 3.6	191.20 ± 1.6	39
	40	256.45 ± 2.5	228.30 ± 1.7	145.94 ± 2.3	54

^a% change in blood glucose was calculated using formula %change = $[(T_c - T_t)/T_c] \times 100$, where T_c = values before administration of drug, T_t = values after administration of drug; data are expressed as mean ± SD; NC = normal control; DC = diabetic control; DGT = diabetic glucophage treated; DTT = diabetic tolbutamide treated.

The spectroscopic data for all the synthesized compounds and coupled products is available in the Supporting Information.

Determination of Solubility. Solubility of all the samples of HES (degraded as well as oxidized) was tested using an aqueous suspension of 500 mg/5 mL distilled water. The suspension was stirred for 15 min at 40 °C, transferred to a centrifuge tube and centrifuged at 1000 rpm for 15 min. The centrifuge tube was removed from the centrifuge machine; the supernatant cleared by centrifugation was carefully transferred into a preweighed crucible and dried in a vacuum oven at 70 °C to a constant weight. The difference in the weight after drying the supernatant gave the weight of the soluble material and was used to calculate the percentage solubility. In the case of coupled products, the increase in the weight of HES was used to calculate the solubility.

Determination of Bioactivity. Healthy growing male albino rats of the Dawley-Sprague family weighing 150–170 g were housed in clean well-ventilated cages at a temperature of 22 ± 1 °C, relative humidity of 40–50%, a constant 12 h light and 12 h dark cycles, free access to standard laboratory animal diet and clean drinking water. The rats were divided into five groups.

Group 1: Normal rats receiving DMSO as vehicle

Group 2: Alloxan induced diabetic control

Group 3: Glucophage treated reference

Group 4: Tolbutamide treated reference

Group 5: Test compound treated rats

The fasting rats (12 h) were given an intraperitoneal (i.p.) injection of alloxan monohydrate freshly dissolved in citrate buffer (pH 4.5) at a dose of 200 mg/kg body weight of the rat

to induce diabetes. Three days later the blood glucose was monitored and rats showing blood glucose levels above 300 mg/dL were taken as diabetic rats for further experiments.

The test compounds were administrated orally to diabetic rats at a varying dose of 20 and 40 mg/kg bodyweight to compare the glucose-lowering potential. An appropriate vehicle control (DMSO) was also run. Blood samples were collected from the tail vein by making a snip with a scalpel or sharp scissors, just prior to drug administration and then at regular intervals of 3 h up to 12 h for blood glucose analysis using Accu-check blood glucose analyzer (Roche). The reduction in blood glucose level (mg/dL) at interval of 3 h was calculated as a difference from the values before drug administration. The percent decrease in the sugar level of normal as well as alloxanized rats was calculated using the formula

$$\% \text{Reduction in blood glucose} = [(T_c - T_t)/T_c] \times 100$$

where T_c = values before administration of drug; T_t = values after administration of drug.

Statistical Analysis. All values presented are means ± SD values. Statistical comparisons of the differences were performed using one way analysis of variance (ANOVA) followed by Tukey's post hoc test, using STATISTICA 5.5. *p*-Values below 0.05 were considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information

All the spectral/characterization data and Representative NMR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Pasut, G., and Veronese, F. M. (2007) Polymer–drug conjugation, recent achievements and general strategies. *Prog. Polym. Sci.* 32, 933–961.
- (2) Marina, Z., Francesca, C., Sara, D., and Gian, M. B. (2011) Multimeric, multifunctional derivatives of poly(ethylene glycol). *Polymers* 3, 1076–1090.
- (3) Torchilin, V. P. (2004) Targeted polymeric micelles for delivery of poorly soluble drugs. *Cell. Mol. Life sci.* 61, 2549–2559.
- (4) Hedlund, B. E. (2000) *Modified polysaccharides exhibiting altered biological recognition*. Bulletin 2000, 39, Eur. Patent 1 037 642 B9.
- (5) Hemberger, J., Orlando, M., Sommermeyer, K., Eichner, W., Frie, S., Lutterbeck, K., Jungheinrich, C., and Scharpf, R. (2009) *Coupling Proteins to a Modified Polysaccharide*. U. S. Patent Appl. 20090233847.
- (6) Franga, D. L., and Harris, J. A. (2000) Polyethylene glycol-induced pancreatitis. *Gastrointest. Endosc.* 52, 789–791.
- (7) Veronese, F. M. (2001) Peptide and protein PEGylation: a review of problems and solutions. *Biomaterials* 22, 405–417.
- (8) Domb, A. J., and Benita, S. (2000) *Conjugates of biologically active substances*. U. S. Patent 6,011,008.
- (9) Langeron, O., Doelberg, M., Ang, E. T., Bonnet, F., Capdevila, X., and Coriat, P. (2001) Voluven, a lower substituted novel hydroxyethyl starch (HES 130/0.4), causes fewer effects on coagulation in major orthopedic surgery than HES 200/0.5. *Anesth. Analg.* 92, 855–862.
- (10) Gandhi, S. D., Weiskopf, R. B., Jungheinrich, C., Koorn, R., Miller, D., Shangraw, R. E., Prough, D. S., Baus, D., Bepperling, F., and Warltier, D. C. (2007) Volume replacement therapy during major orthopedic surgery using Voluven (hydroxyethyl starch 130/0.4) or hetastarch. *Anesthesiology* 106, 1120–1127.
- (11) Jungheinrich, C., Sauermann, W., Bepperling, F., and Vogt, N. H. (2004) Volume efficacy and reduced influence on measures of coagulation using hydroxyethyl starch 130/0.4 (6%) with an optimized in vivo molecular weight in orthopaedic surgery: a randomised, doubleblind study. *Drugs R&D* 5, 1–9.
- (12) Yamakage, M., Bepperling, F., Wargenau, M., and Miyao, H. (2012) Pharmacokinetics and safety of 6% hydroxyethyl starch 130/0.4 in healthy male volunteers of Japanese ethnicity after single infusion of 500 mL solution. *J. Anesth.* 26, 851–857.
- (13) Jungheinrich, C., Scharpf, R., Wargenau, M., Bepperling, F., and Baron, J. F. (2002) The pharmacokinetics and tolerability of an intravenous infusion of the new hydroxyethyl starch 130/0.4 (6%, 500 mL) in mild-to-severe renal impairment. *Anesth. Analg.* 95, 544–551.
- (14) Abbas, M. A., Hameed, S., and Kressler, J. (2013) Selective degradation, oxidation, and characterization of hydroxyethyl starch for potential use as a drug carrier. *Starch/Stärke* 65, 264–272.
- (15) Ahmad, I., Hameed, S., Duddeck, H., Lenzen, S., Rustenbeck, I., and Ahmad, R. (2002) *N*-Arylsulfonylbenzimidazolones as potential hypoglycemic agents. *Z. Naturforsch.* 57b, 349–354.
- (16) Turconi, M., Nicola, M., Qunitero, M. G., Maiocchi, L., Micheletti, R., Giraldo, E., and Donetti, A. (1990) Synthesis of a new class of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylic acid-derivatives as highly potent 5-HT₃ receptor antagonists. *J. Med. Chem.* 33, 2101–2108.
- (17) Howard, H. R., Sarges, R., Siegel, T. W., and Beyer, T. A. (1992) Synthesis and aldose reductase inhibitory activity of substituted 2(1H)-benzimidazole-1-acetic acids and oxindole-1-acetic acids. *Eur. J. Med. Chem.* 27, 779–789.
- (18) Bianchi, M., Butti, A., Rossi, S., Barzaghi, F., and Marcara, V. (1983) Compounds with antiulcer and antisecretory activity. 2. 3-heteroaryl-benzimidazolin-2-ones. *Eur. J. Med. Chem.* 18, 495–500.
- (19) McKay, M. C., Dworetzky, S. I., Meanwell, N. A., Olesen, S. P., Reinhart, P. H., Levitan, I. B., Adelman, J. P., and Gribkoff, V. K. (1994) Opening of large-conductance calcium-activated potassium channels by the substituted benzimidazole NS004. *J. Neurophysiol.* 71, 1873–1882.
- (20) Derand, R., Bulteau-Pignoux, L., and Becq, F. (2003) Comparative pharmacology of the activity of wild-type and G551D mutated CFTR chloride channel: Effect of the benzimidazolone derivative NS004. *J. Membr. Biol.* 194, 109–117.
- (21) Weidhase, R., and Faude, K. (1998) Hydroxyethyl starch – an interim report. *Anaesthesiol. Reanim.* 23, 4–14.
- (22) Iqbal, Z., Ali, S., Iqbal, J., Abbas, Q., Qureshi, I. Z., and Hameed, S. (2013) Dual action spirobicycloimidazolidine-2,4-diones: Antidiabetic agents and inhibitors of aldose reductase-an enzyme involved in diabetic complications. *Bioorg. Med. Chem. Lett.* 23, 488–491.
- (23) Iqbal, Z., Akhtar, T., Hendsbee, A. D., Masuda, J. D., and Hameed, S. (2012) Synthesis, characterization, and hypoglycemic activity of 3-(arylsulfonyl)spiroimidazolidine-2,4-diones. *Monatsh. Chem.* 143, 497–504.
- (24) Hussain, A., Kashif, M. K., Naseer, M. M., Rana, U. A. Hameed, S. (2014) Synthesis and in vivo hypoglycemic activity of new imidazolidine-2,4-dione derivatives. *Res. Chem. Intermed.* In press, DOI 10.1007/s11164-014-1814-3.
- (25) Van den Branden, S., Compennolle, F., and Hoornaert, G. J. (1992) Mimicking of ergot alkaloids and synthetic piperidine drugs by 2,5-substituted piperidines derived from cis and trans ethyl 1-benzyl-6-cyano-3-piperidinecarboxylate. *Tetrahedron* 48, 9753–9766.
- (26) Haque, M. R., and Rasmussen, M. (1994) Ambident heterocyclic reactivity: Alkylation of 2-substituted-4-methylbenzimidazoles. *Tetrahedron* 50, 5535–5554.
- (27) Meanwell, N. A., Sit, S. Y., Gao, J., Wong, H. S., Gao, Q., Laurent, D. R. S., and Balasubramanian, N. (1995) Regiospecific functionalization of 1,3-dihydro-2H-benzimidazol-2-one and structurally related cyclic urea derivatives. *J. Org. Chem.* 60, 1565–1582.
- (28) Tserng, K.-Y., and Bauer, L. (1973) Novel Lossen rearrangements of 3-benzenesulfonyloxy(1H- and 1-methyl)-2,4-quinazoline-diones induced by alkoxide ions. *J. Org. Chem.* 38, 3498–3502.
- (29) Wuts, P. G. M., and Greene, T. (1991) *Protective Groups in Organic Synthesis*, 2nd ed.; John Wiley and Sons, New York.
- (30) Abbas, M. A., Hameed, S., and Kressler, J. (2013) Preparation of 2(3H)-benzimidazolone and its derivative under aqueous condition as a potential agent for antidiabetic compounds. *Asian J. Chem.* 25, 509–511.