Research Article

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Benzimidazole chalcones: Potent glycosidase inhibitors

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ABSTRACT

Benzimidazole-2-substituted chalcones, a new class of glycosidase inhibitors are synthesized using recyclable PEG-400 as an alternative reaction solvent. Effect of synthesized compounds on glycosidase enzymes (α-amylase and glucoamylase) were studied *in vitro*. Chalcones bearing electron withdrawing groups exhibit strong a-glycosidase inhibitory action whereas electron donating groups displayed moderate inhibitory action. However, nitro derivative of chalcones **3d** (IC_{50} = 1 mM), **3m** (IC_{50} = 2 mM) and **3n** (IC_{50} = 4.9 mM) proved to be potent inhibitors against α-amylase whereas derivative **3h** (IC_{50} = 19 mM) was found to be moderate inhibitor of glucoamylase.

Keywords: α-glycosidase inhibitors, Benzimidazole chalcones, PEG-400, postprandial hyperglycemia.

INTRODUCTION

Diabetes mellitus (hyperglycemia) is one of the most chronicle disease widely speeded in the population with increase in age and obesity level. Recent studies have also witnessed excess stress and depression to be one of the major reasons which lowers the body metabolism and disturbs the insulin secretion which regulates the required sugar level in the body thereby causing diabetes[1, 2]. The post advances of this disease lead to severe macro-vascular complications. However to negotiate the normal blood glucose level in the body becomes more intriguing for the diabetes patients[3, 4]. Several commercial drugs are available against hyperglycemia, nevertheless amalgamation of dietary constraints with habitual exercise line up and proper medication could be the best way to control the postprandial plasma glucose level[5, 6]. Few therapeutic approaches are put forth for decreasing the postprandial hyperglycemia by lowering the glucose absorption by the inhibition of carbohydrate hydrolyzing enzymes, viz. a-amylase (EC 3.2.1.1) and a-glucoamylase (EC 3.2.1.20) in the digestive system[7, 8]. These enzymes during the digestive processes catalyses the initial step of starch hydrolysis to maltose followed by the final conversion step of starch into glucose. Hence, glycosidase inhibitors can hamper/retard the decomposition and absorption of dietary carbohydrates to suppress postprandial hyperglycemia and may be useful to treat diabetic patients[9, 10, 11]. Some inhibitors viz. acarbose, miglitol and voglibose are currently relevant in clinical to inhibit glycosidases[12, 13]. However, many of them have their limitations such as non-specificity, producing serious gastrointestinal disorders viz. bloating, abdominal discomfort, diarrhea and flatulence[14]. Thus, developing novel glycosidase inhibitors for the treatment against diabetes has become a keen interest of our research.

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According to recent findings, chalcones are found to act as new precursors of highly specific glycosidase inhibitors[15]. Natural and synthetic chalcones (1, 3-diaryl-2-propen-1-ones) with an enone system between two aromatic rings, exhibit a broad spectrum of interesting pharmacological and biological activities such as Antileishmanial[16], Antimalarial[17], Antiplasmodial[18], Anti-inflammatory[19], Anti-cancer[20], and Anti-HIV[21].

To the best of our knowledge to date, benzimidazole chalcone derivatives have not been screened as glycosidase inhibitors. Hence, in the present study, we have synthesized benzimidazole chalcones and evaluated their *in vitro* study on a-amylase and glucoamylase.

MATERIALS AND METHODS

General

Completion of the reaction was monitored by thin layer chromatography (TLC) using glass plates coated with silica gel. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance spectrometer 300MHz. All ^{1}H and ^{13}C NMR samples were run in DMSO without TMS and $CDCl_3$ with 1% TMS respectively. Chemical shifts are expressed as d. IR spectra were obtained on Perkin Elmer FT-IR spectrophotometer spectrum 2000 using potassium bromide (KBr) pellets. Mass spectra were obtained on Thermo-Finnigm Discovery-Max GC-MS. All the melting points were determined in an open capillary tube using Expo Hi Tech. melting point apparatus. Enzyme α-amylase (source: ex-porcine) and glucoamylase (source: aspergillus niger) were obtained from SRL and Himedia Pvt. Ltd., India respectively. All experiments were performed in 3 different sets, with each set in triplicates.

General Procedure of Benzimidazole-2-substituted chalcones

An equimolar mixture of 2-acetyl benzimidazole **1** (1 mmol), aromatic aldehydes **2** (1 mmol) and NaOH (1mmol) was stirred in PEG-400 (15 mL) at 40°C for 2 hours (Scheme 1) After completion of the reaction (as monitored by TLC) the crude mixture was worked up in ice-cold

water (100 mL), neutralized with dilute HCl. The separated out product was filtered. The filtrate was then evaporated to remove excess water leaving PEG. The same PEG was utilized to synthesize further chalcones.

The spectral data of some representative compounds are as follows:

1-(1H-Benzimidazole-2-yl)-3-Phenyl-2-Propen-1-one (3a)
m. p. 195-197° C; Yield: 82 %, FT-IR (KBr), υ_{max} cm^{-1}: 3251 (-NH), 1661 (>C=O), 1595 (-C=N), 1330 (C-N); ^{1}H NMR (DMSO, TMS, 300 MHz): 13.49 (1H, s), 8.13 (1H, d, J= 16.2 Hz), 7.30-7.98 (9H, m); ^{13}C NMR ($CDCl_3$, TMS, 300 MHz): 181.4, 149.4, 144.7, 143.4, 135.2, 134.7, 131.5, 129.6(2C), 129.3(2C), 126.2, 123.6, 121.9, 121.6, 113.3; MS: m/e 248 (M^+).

1-(1H-Benzimidazole-2-yl)-3-(4-Chloro Phenyl)-2-Propen-1-one (3b)
m. p. 200-202° C; Yield: 80 %, FT-IR (KBr), υ_{max} cm^{-1}: 3277 (-NH), 1662 (>C=O), 1591 (-C=N), 1330 (C-N), 740 (C-Cl); ^{1}H NMR (DMSO, TMS, 300 MHz):13.50 (1H, s), 8.13 (1H, d, J= 16.2 Hz), 7.32-7.96 (9H, m); ^{13}C NMR ($CDCl_3$, TMS, 300 MHz): 181.3, 149.3, 143.4, 143.2, 136.0, 135.2, 133.6, 131.5, 131.0, 129.6, 129.1, 126.3, 123.7, 122.7, 121.6, 113.3; MS: m/e. 282 (M^+), 284 (M^{+2}).

1-(1H Benzimidazole-2-yl)-3-(3, 4-dimethoxy Phenyl)-2-Propen-1-one (3f)
m. p. 192-194° C; Yield 85 %, FT-IR (KBr), υ_{max} cm^{-1}: 3257 (-NH), 1653 (>C=O),1575 (-C=N), 1419 (C-N), 1267(C-O-C); ^{1}H NMR (DMSO, TMS, 300 MHz): 13.44 (1H, S), 8.0 (1H, d, J=16.2 Hz), 7.03-7.97 (8H, m), 3.86 (3H, s), 3.82 (3H, s); ^{13}C NMR ($CDCl_3$, TMS, 300 MHz): 181.3, 152.2, 149.6, 149.5, 145.3, 143.5, 135.1, 127.5, 126.0, 124.5, 123.5, 121.4, 119.5, 113.2, 112.2, 111.2; MS: m/e 308 (M^+).

1-(1H-Benzimidazole-2-yl)-3-(thiophene-2-yl)-2-propen-1-one (3h)
m. p. 210-212° C; Yield 83 %, FT-IR (KBr), υ_{max} cm^{-1}: 3255 (-NH), 1651 (>C=O),1575 (-C=N), 1321 (C-N); ^{1}H NMR (DMSO, TMS, 300 MHz): 13.47 (1H, s), 8.16 (1H, d, J= 16.2 Hz), 7.20-7.85 (8H, m); ^{13}C NMR ($CDCl_3$, TMS, 300 MHz): 180.8, 149.3, 140.1, 137.4, 134.6, 131.5, 129.5, 126.2, 123.6, 121.6, 120.3, 113.2; MS: m/e 254 (M^+).

1-(1H-Benzimidazole-2-yl)-3-(4-Cyno Phenyl)-2-Propen-1-one (3l)
m. p. 225-227° C; Yield 76 %, FT-IR (KBr), υ_{max} cm^{-1}: 3261 (-NH), 2227 (Aromatic-CN), 1663 (>C=O), 1660 (-C=N), 1326 (C-N); ^{1}H NMR (DMSO, TMS, 300 MHz): 13.47 (1H, s), 8.16 (1H, d, J= 16.2 Hz), 7.20-7.85 (8H, m); ^{13}C NMR ($CDCl_3$, TMS, 300 MHz): 180.8, 149.3, 140.1, 137.4, 134.6, 131.5, 129.5, 126.2, 123.6, 121.6, 120.3, 113.2; MS: m/e 254 (M^+).

Assay of a**-amylase:**
0.5 mL of the reaction mixture containing 0.1 mL modulator, 0.3 mL of 20mM phosphate buffer (pH 4.5), and 0.1 mL of a-amylase (1.3µg) were incubated at 37° C for 30 minutes. Then 0.5 mL of starch solution (10 mg/mL prepared in 20mM phosphate buffer pH 7.0) was added and incubated further at 37° C for 30 minutes. The reaction was then terminated keeping the test tubes in boiling water bath for 1-2 minutes, cooled under running tap water. 1mL of DNS (3, 5-Dinitro Salicylic Acid) was added and the test tubes were kept in boiling water bath for 15 min. Then the test tubes were cooled and diluted with 8mL distilled water. Acarbose was used as standard. The absorbance was recorded at 530 nm using spectrophotometer and liberated glucose was then estimated. A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute.

Assay of glucoamylase:
0.5 mL of the reaction mixture containing 0.1 mL modulator, 0.3 mL of 100mM acetate buffer (pH 4.5), and 0.1 mL of glucoamylase (1.3µg) were incubated at 37° C for 30 minutes. Then 0.5 mL of starch solution (5 mg/mL prepared in 100mM acetate buffer pH 4.5) was added and incubated further at 37° C for 30 minutes. The reaction was then terminated by keeping the test tubes in boiling water bath for 1-2 minutes, cooled under running tap water. 2mL of DNS (3, 5-Dinitro Salicylic Acid) was added and the test tubes were kept in boiling water bath for 15 min. Then the test tubes were cooled and diluted with 7mL distilled water. Acarbose was used as standard. The absorbance was recorded at 530 nm using spectrophotometer and liberated glucose was estimated. A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute.

The maximum inhibition was determined from plots of percent inhibition versus modulator (where concentration of modulator was taken 40 mM) and calculated as below.
% activity= (enzyme activity of test / enzyme activity of control) x 100;
% inhibition = (100 -% activity)

RESULTS AND DISCUSSION
Enzyme a-amylase and glucoamylase were used as target enzymes for screening the above mentioned analogues (3a-n) for their inhibitory activities. The control represents 100 % enzyme activity for a-amylase and glucoamylase. The activity obtained for each compound was normalized to percent activity from which the percent inhibition was calculated. (Table 2)

Compounds **3a**, **3b**, **3d**, **3g**, **3h**, **3i**, **3j**, **3l**, **3m** and **3n** were found to exhibit more than 50 % inhibition while analogs **3c**, **3f** and **3k** showed less than 50 % inhibition against α-amylase. On the other hand, analogs **3b**, **3d**, **3h**, **3i**, **3j** and **3m** displayed 51% inhibition and rest of the analogues showed less than 50 % inhibition against glucoamylase.

Amongst the known glycosidase inhibitors, acarbose is chosen as the standard reference. Those compounds exhibiting maximum inhibition for α-amylase and glucoamylase were taken as lead compounds and were further tested for $IC_{50.}$

The scope of the present work meets our investigation of synthesizing the benzimidazole chalcone derivatives as potent inhibitors of α-amylase and glucoamylase *in vitro*. From the results, it is evident that benzimidazole chalcones containing electron withdrawing groups in C ring are potent inhibitors of α-amylase and moderate inhibitors of glucoamylase. Whereas, Benzimidazole chalcones containing elec

Table 1: Physical constant for the synthetic compounds

Compd.	R	Molecular formula	M. W.	Yield[a] %	Melting point °C Found	Reported[ref]
3a	Phenyl	$C_{16}H_{12}N_2O$	248	82	195-197	194-196[22]
3b	4-chlorophenyl	$C_{16}H_{11}ClN_2O$	282	80	200-202	202-204[22]
3c	4-methoxyphenyl	$C_{17}H_{14}N_2O_2$	278	79	185-187	184-188[22]
3d	4-nitro phenyl	$C_{16}H_{11}N_3O_3$	293	70	243-245	—-
3e	4-dimethylaminophenyl	$C_{18}H_{17}N_3O$	291	78	273-275	—-
3f	3,4-dimethoxyphenyl	$C_{18}H_{16}N_2O_3$	308	83	192-194	182-184[22]
3g	2-cholrophenyl	$C_{16}H_{11}ClN_2O$	282	75	218-220	—-
3h	Thiophene-2	$C_{14}H_{10}N_2OS$	254	83	210-212	—-
3i	4-methylphenyl	$C_{17}H_{14}N_2O$	262	80	207-209	—-
3j	4-bromophenyl	$C_{16}H_{11}BrN_2O$	327	79	220-222	224-226[22]
3k	5-bromo-thiophene-2	$C_{14}H_9BrN_2OS$	333	85	237-239	—-
3l	4-cynophenyl	$C_{17}H_{11}N_3O$	273	76	225-227	—-
3m	3-nitro phenyl	$C_{16}H_{11}N_3O_3$	293	80	211-213	—-
3n	4-chloro-3-nitrophenyl	$C_{16}H_{10}ClN_3O_3$	327	74	209-211	—-

[a]Yields refers to pure isolated products.All the products are known and were characterized by spectral data (IR, [1]H NMR, [13]C NMR and GC-MS) and melting points compare with reference.

Table-2: Inhibitory activity of benzimidazole-2-substituted chalcones (3a-j) on a-amylase and glucoamylase activity.

Modulator*	a –Amylase Activity (U)	% Inhibition	Glucoamylase Activity (U)	% Inhibition
Control	32.50	0.00	3.97	0.00
3a	06.67	79.49	2.31	41.87
3b	12.90	60.26	1.79	54.79
3c	16.70	48.72	2.56	35.41
3d	01.67	94.87	1.79	54.79
3e	32.50	0	3.97	0
3f	26.30	19.23	2.82	28.95
3g	10.80	66.67	2.31	41.87
3h	05.00	84.62	1.41	64.48
3i	14.60	55.13	1.92	51.56
3j	06.67	79.49	1.40	64.48
3k	16.70	48.72	3.46	12.81
3l	11.70	64.10	2.82	28.95
3m	01.67	94.87	1.92	51.56
3n	0.83	97.44	2.31	41.87
Acarbose	07.50	76.90	0.69	82.50

**Concentration of Modulator is 40mM.The data is indicated as the mean ± SEM (n = 3).*

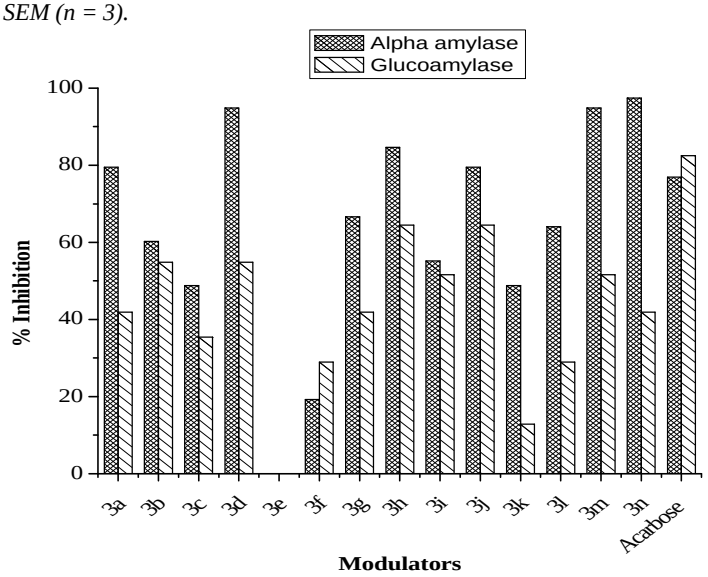
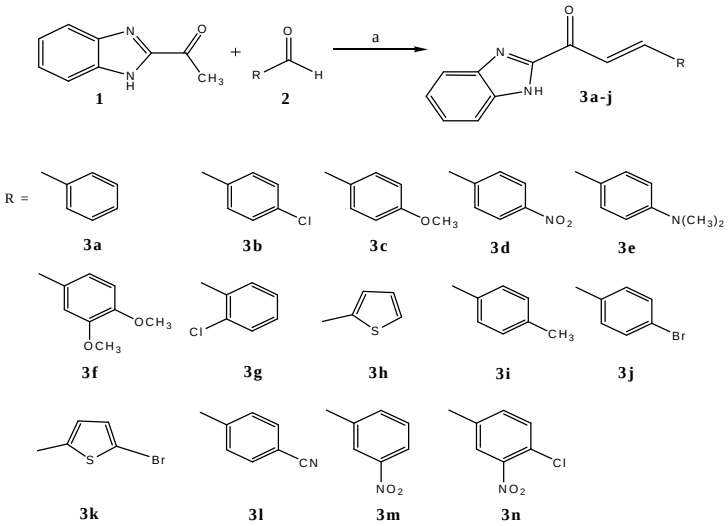


Figure 1: Enzyme activity after inhibition with benzimidazole-2-substituted chalcones (3a-j) on a-amylase and glucoamylase. Acarbose is taken as standard. The data is indicated as the mean ± SEM (n = 3). Concentration of modulator is 40mM.



Scheme-1 Synthetic route of benzimidazole-2-substituted chalcone analogues: (a) NaOH, PEG-400, 40° C.

tron donating groups in C ring shows moderate inhibition of a-amylase and comparably lower inhibition of glucoamylase.

At the concentration of 40 mM, amongst the various analogs **3d, 3h, 3m** and **3n** were found to exhibit excellent inhibition against a-amylase (94.87 %, 84.62 %, 94.87 and 97.44 % respectively) greater than reference standard acarbose (76.90 %). At this concentration, analog **3n** showed maximum inhibition (97.44 %) as compared to the rest of the analogs. Analogs **3d** and **3m** bears nitro substituent at para and meta position in C ring respectively, while, **3n** contains nitro group at meta position with chloro group at para position. Analogs **3h** and **3j** showed moderate inhibition (64.48 % and 64.48 % respectively) against glucoamylase as compared to reference standard acarbose (82.50 %). Analog **3e** was found to be inactive against both the glycosidases. (Table 2 and Fig. 1)

Inspired by the outstanding results obtained from the above analysis, these compounds were further tested for IC_{50} which inferred that α-amylase inhibitory activities of benzimidazole chalcones, **3d** (IC_{50} = 1 mM), **3m** (IC_{50} = 2 mM) and **3n** (IC_{50} = 4.9 mM) were found to be

effective inhibitors in comparison with compound **3h** (IC_{50} = 18 mM). The nitro derivatives of benzimidazole chalcones act as potent inhibitors and rest of the analogues show moderate inhibitory activity against α-amylase. Similarly, Results revealed that, Compound **3h** (IC_{50} = 19 mM) is more effective than 3**j** (IC_{50} = 22 mM) against glucoamylase. Results evidently prove that compound **3d** is by far the most effective inhibitor of α-amylase.

CONCLUSION

Our synthetic investigation offers a remarkable use of eco-friendly solvent in simple and mild reaction conditions, and thereby yielding benzimidazole chalcones, which act as potent inhibitors of a-amylase and moderate inhibitors of glucoamylase.

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REFERENCES

1. Alberti KG, Zimmet PZ, Definition, diagnosis and classification of diabetes mellitus and its complications Part 1: diagnosis and classification of diabetes mellitus. Report of a WHO Consultation, Diabetic Medicine, 1998, 15, 539–553.
2. Kim YM, Wang MH, Rhee HI, A novel alpha-glucosidase inhibitor from pine bark, Carbohydrate Research, 2004, 339, 715-717.
3. Mooradian AD, Thurman JE, Drug therapy of postprandial hyperglycaemia, Drugs, 1999, 57, 19-29.
4. Kim JS, Kwon CS, Son KS. Inhibition of alpha-glucosidase and amylase by luteolin a flavonoid, Biosci. Biotechnol. Biochem, 2000, 64, 2458-2461.
5. Jarvinen YH, Acute and chronic effects of hyperglycemia on glucose metabolism, Diabetologia, 1990, 33, 579–585.
6. Goke B, Herrmann-Rinke C, The evolving role of alpha-glucosidase inhibitors. Diabetes, Metab. Rev., 1998, 14, 1S31–1S38.
7. Lebovitz HE, Alpha-glucosidase inhibitors, Endocrine Metab. Clin., 1998, 26, 539-551.
8. Eichler HG, Korn A, Gasic S, The effect of a new specific *a*-amylase inhibitor on post-prandial glucose and insulin excursions in normal and Type 2 (non-insulin dependent) diabetic patients, Diabetologia, 1984, 26, 278–281.
9. Braun C, Brayer GD, Withers SG, Mechanism-Based Inhibition of Yeast Alpha-Glucosidase and Human Pancreatic Alpha-Amylase by a New Class Of Inhibitors: 2-deoxy-2,2-difluoro Alpha-Glycosides, J. Biol. Chem., 1995, 270, 26778-26781.
10. Begovic ME, New potent alpha-glucohydrolase inhibitor MDL 73945 with long duration of action in rats, Diabetes, 1991, 40, 825-830.
11. Mehta A, Zitzmann N, Rudd PM, Block TM, Dwek RA, Alpha-glucosidase inhibitors as potential broad based antiviral agents, FEBS Lett., 1998, 430, 17-22.
12. Fernandes B, Sagman U, Auger M, Demetrio M, Dennism JW, Beta 1-6 branched oligosaccharides as a marker of tumor progression in human breast and colon neoplasia, Cancer Res, 1991, 51, 718-723.
13. Matsuo T, Odaka H, Ikeda H, Effect of an intestinal disaccharidase inhibitor (AO-128) on obesity and diabetes, Am. J. Clin. Nutr., 199, 55, 314s-317s.
14. Cheng YY, Fantus IG, Oral antihyperglycemic therapy for type 2 diabetes mellitus, Canadian Medicinal Association Journal, 2005, 172, 213-226.
15. Mahmoud N, Azadeh EH, Nastaran H, Parichehreh Y, Kazem P, Bagher L, Trans-chalcone: a novel small molecule inhibitor of mammalian alpha-amylase, Mol. Biol. Rep., 2011, 38, 1617-1620.
16. Nielsen SF, Christensen SB, Cruciani G, Kharazmi A, Liljefors TJ, Antileishmanial chalcones: Statistical design, synthesis and three-dimentional quantitative structure-activity relationship analysis, Med. Chem., 1998, 41, 4819-4832.
17. Li R, Kenyon G L, Cohen F E, et al, In Vitro Antimalarial Activity of Chalcones and Their Derivatives, J. Med. Chem., 1995, 38, 5031-5037.
18. Liu M, Wilairat P, Go ML, Antiplasmodial Chalcones Inhibit Sorbitol-Induced Hemolysis of Plasmodium falciparum-Infected Erythrocytes, J. Med. Chem., 2001, 44, 4443-4452.
19. Hsieh HK, Lee TH, Wang JP, Wang JJ, Lin CN, Synthesis and anti-inflammatory effect of chalcones and related compounds, Pharm. Res., 1998, 15, 39-46.
20. Ducki S, Forrest R, Hadfield JA, Kendall A, Lawrence NJ, McGown AT, Rennison D, Potent Antimitotic and Cell Growth Inhibitory Properties of Substituted Chalcones, Bioorg. Med. Chem. Lett., 1998, 8, 1051-1060.
21. Jiu-Hong Wu, Wang Xi-Hong, Yang-Hua Yi, Kuo-Hsiung Lee, Anti-AIDS agents 54. A potent anti-HIV chalcone and flavonoids from genus Desmos, Bioorg. Med. Chem., 2003, 13, 1813-1815.
22. Mohammad S, Abdullah MM, Bakht MA, Jaseela M, Pyrazoline bearing benzimidazoles: Search for anticancer agent, European Journal of Medicinal chemistry, 2010, 45, 114-119.

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