Section: Research Paper

Bosentan Effect on Glimepiride's Antidiabetic Effect in Animal Model

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Abstract:

Due to a variety of comorbidities, Patients with type 2 diabetes usually require a multifaceted approach to therapy. A large number of medications taken at the same time increases the risk of undesirable drug effects or drug interactions in the patient. It's vital to think about cytochrome P-450 (CYP) enzyme interactions while using a multifactorial pharmacotherapy approach. The cytochrome P450 enzymes CYP2C9 and CYP3A4 metabolise bosentan in the liver Similarly, glimepiride is metabolised in the liver by CYP2C9 to the active M_1 (hydroxyl) metabolite and then to inactive M_2 (carboxy) metabolite. This study was conducted to investigate a possible pharmacokinetic interaction between bosentan and glimepiride. Interaction of glimepiride, the known second-generation, long-acting insulin secretagogues agent with Bosentan, a pulmonary antihypertensive agent, In healthy and alloxan-induced diabetic rats, was tested. Blood samples were taken from rats at various intervals up to 24 hours and blood glucose levels were calculated. The parameters considered for the analysis of the effect of glimepiride-induced hypoglycemia were the onset of hypoglycemia, duration of hypoglycemia (duration of time in which more than 15 % - 20 % decrease in blood glucose level is managed to maintained), and peak hypoglycemia. In both healthy albino rats and diabetic rats, a single dose of bosentan did not affect blood glucose levels. These results suggest that bosentan has no hypoglycemic effect, implying that the drug-drug interaction with glimepiride is of the pharmacokinetic kind.

Keywords: Bosentan, glimepiride, pharmacokinetic interaction, drug-drug interaction, hypoglycaemia.

INTRODUCTION:

A drug interaction happens when a drug alters a drug's action, causing the effects to be increased or lessened, or developing a new effect that none of the medications could induce on their own. Drug interactions can be either useful or harmful to humans, but it has been discovered that the negative impacts of drug interactions exceed the positive effects. It has the potential to change a drug's diagnostic, preventive or therapeutic function. [1] There is a category of drugs that interacts and interferes with the action of diabetes medications. [2], [3], [4], [5] Hence, it is important to monitor patients' glucose levels carefully. [6], [7] Diabetes complications are the major cause of morbidity and mortality among diabetics. [8], [9] The endothelin-1 (ET) receptor antagonist bosentan is an important drug for the efficient therapy of pulmonary arterial hypertension patients. Bosentan is primarily metabolised by the cytochrome P450 (CYP) 3A4 and 2C9 enzymes, with numerous transporters involved in hepatic absorption and biliary excretion. [10] Oral hypoglycemic medications are used to treat type 2 diabetes, and glimepiride is one of them. It's a second-generation sulphonyureas widely used in the management of T2DM as a secretagogue and it's metabolised by the CYP2C9 isoenzyme. [11, 12]

As a result, the goal of this study is to learn more about the potential drug-drug interactions among hypoglycemic medications like glimepiride and pulmonary antihypertensive drugs such as bosentan in both healthy and diabetic rats.

Material and methods:

The research was conducted in our institution's Department of Pharmacology, which is properly licenced by CPCSEA (Committee for Control and Supervision of Experiments in Animals). The Institution Animal Ethics Committee accepted the study protocols for experiments in experimental animals following current CPCSEA standards.

Animals:

The rats used were all procured from LACSMI BioFarms in Ale Phata, Pune. The animals were kept in the institutional animal house under standard husbandry conditions. The current investigation included a total of 12 male and female rats.

Common Experimental Techniques:

Method for oral administration: [13]

A 1 mL glass syringe and an oral feeding needle were used for oral feeding (bought from Space Labs in Nashik). The oral feeding needle was directly inserted in the oesophagus of the rat, after that, we gently press the plunger for the administration of medicine, and then some water was added to confirm that the accurate quantity of medicine was administered.

Method for blood sampling: [14, 15, 16]

We gave small anaesthesia to rats and after that, we place them on the operating table, squeezing the tail with the help of Xylene to widen the rat's vein and cut the tail tip, and collected blood in the epindroff tubes which contain an anticoagulant combination.

Blood glucose estimation:

GOD/POD technique, developed by Trinder in 1964, is an example of an evolved method [17]. This procedure is straightforward, one-step, quick, accurate, and precise enough. The use of a glucometer to test blood glucose is a simple, quick, and cost-effective way of glucose monitoring. On the other hand, despite the longer operational time and greater cost, centralised laboratory glucose testing is still a more reliable tool for patient diagnosis and management [18]. As a result, this strategy was used in the current investigation.

EXPERIMENTAL PROCEDURE:

Impact of Bosentan Pretreatment on Glimepiride's hypoglycemic action in healthy albino rats:

For this experiment, we selected a total of six albino rats of both sexes, 150 to 180 grams in weight, who were chosen for the experiment. They were well-labelled for easy identification purposes. The rats were kept in standard husbandry settings in cages. The food was taken out 18 hours before the start of the experiment. Water, on the other hand, was available at all times. The fasting was continued until the end of the experiment. From the tail vein, the blood samples (0.5 ml each) were taken on the next day to determine the basal glucose levels. Following that, In the first part of the experiment, the animals were given a suspension of glimepiride 0.41 mg/kg via the oral route. With the time intervals of 0, 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 hours the blood was collected from the tail vein and analysed the blood glucose concentrations using the GOD/POD methodology. The rats in the second part of the experiment were given a dose of Bosentan 10 mg/kg suspension orally for 7 consecutive days and on the 7^{th} day after Bosentan, they fasted for 18 hours. This fasting was kept up until the end of the tests. Water, on the other hand, was available at all times. Glimepiride 0.41 mg/kg, was given to the same rats on the eighth day, one hour following the administration of bosentan 10 mg/kg. Following that, blood samples were obtained from the tail vein at the same time intervals and analysed using the GOD/POD method to determine blood glucose concentration.

Impact of Bosentan Pretreatment on Glimepiride's hypoglycemic action in diabetic rat:

Yet, it was unclear whether bosentan shows some effect on anti-diabetic medications within pathophysiological circumstances such as diabetes mellitus. As a result, in order to clarify this point, we plan to use diabetic rats as experimental animals in the current study. Pre-treatment with bosentan (10 mg/kg) was found to have a substantial effect on the peak hypoglycemia produced by glimepiride 0.41 mg/kg in the earlier phase of this experiment. As a result, the dose of Bosentan was determined to confirm the interactions in diabetic albino rats.

Induction of diabetes [19, 20]:

For diabetes induction, albino rats of both males and females were utilized. For two days, these animals were given a dose of 100 mg/kg and 50 mg/kg of body weight respectively of a newly produced aqueous solution of alloxan monohydrate were injected intraperitoneally. Then, to counteract the initial hypoglycemia, 10% dextrose was given. Blood sugar levels were assessed, and rats with more than 250 mg/dL of fasting blood sugar levels were chosen for the experiment.

Experimental procedure:

During the experiment, male and female diabetic rats (n=6), with blood glucose levels of more than 250 mg/dl were selected, once 48 hours of administration of alloxan. The selected rats fasted for 18 hours in advance at the beginning of the experiment. Unlimited access to water was given during the experiment. During the study, fasting was maintained. To calculate the fasting blood glucose levels, the blood samples were taken and analyzed. Then, the rats received an oral suspension of glimepiride 0.41 mg/kg and the GOD/POD method was used to estimate blood glucose levels after blood samples were taken at regular intervals. While in the following portion of the experiment, the rats were then given bosentan (10 mg/kg) for seven consecutive days. On the seventh day, after 6 hrs of bosentan administration, the rats fasted for 18 hrs. On the next day, oral administration of bosentan 10 mg/kg was done and after 60 minutes, glimepiride 0.41 mg/kg was given to the same animals. Following that, samples of blood were obtained at regular intervals and analysed using the GOD/POD method. The levels of blood glucose were measured in milligrams/dilutions.

Results:

The parameters considered for the evaluation of influence on glimepiride-induced hypoglycemia were the onset of hypoglycemia (time taken by a drug to reduce blood glucose level to the extent of 15 % -20 %), duration of hypoglycemia (time duration in which more than 15 % -20 % reduction in blood glucose level is maintained), and peak hypoglycemia.

Impact of Bosentan Pre-Treatment on Glimepiride Hypoglycemic Effect in Healthy Albino Rats:

In this study, it is found that the pretreatment of bosentan (10 mg/kg for seven days) had significantly altered the onset of hypoglycemia from 1 hour i.e. 17.14 ± 0.55 % reduction before treatment to 2 hours i.e. 15.39 ± 0.50 % reduction after treatment. however, it did affect peak hypoglycemia significantly i.e. (47.25 ± 0.62 % reduction before treatment to 34.97 ± 0.62 % reduction after treatment at the 8^{th} hour. Though, the duration of hypoglycemia was 24 hours and reduced to 18 hours only after treatment i.e. (17.90 ± 0.41 % reduction before treatment and 16.13 ± 0.51 % reduction after treatment. Table No.1 summarizes the results, which are schematically presented in Figure No. 1.

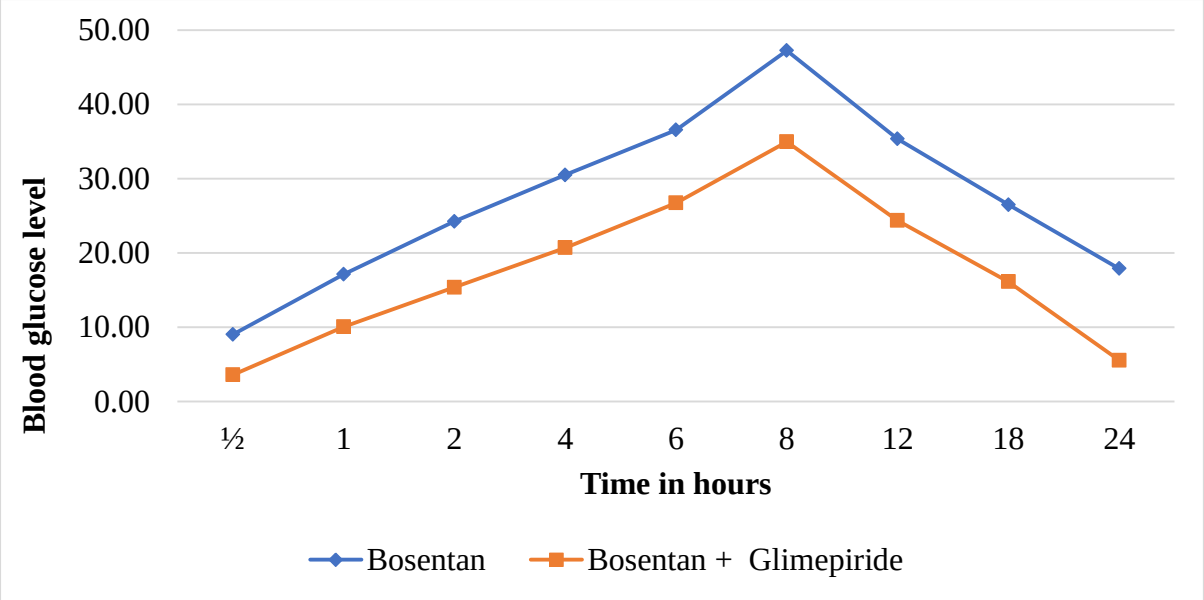
Table No. 1: The percentage levels of blood glucose in healthy albino rats earlier and later therapy of bosentan (10 mg/kg) on glimepiride (0.41 mg/kg).

Time in Hrs	Glimepiride blood glucose levels in percentage (mg/dl)	Bosentan blood glucose levels in percentage (mg/dl)	Glimepiride + Bosentan blood glucose levels in percentage (mg/dl)
	Mean ± SEM	Mean ± SEM	Mean ± SEM
0	-	-	-
½	9.02±0.51	2.50±0.11	3.60±0.26***
1	17.14±0.55	6.49±0.13	10.07±0.55***
2	24.26±0.45	8.94±0.12	15.39±0.50***
4	30.50±0.68	11.53±0.08	20.72±0.51***
6	36.58±0.73	13.25±0.25	26.74±0.54***
8	47.25±0.62	8.48±0.14	34.97±0.62***
12	35.37±0.68	5.41±0.11	24.38±0.53***

18	26.50±0.67	2.36±0.09	16.13±0.51***
24	17.90±0.41	1.07±0.16	5.56±0.61***

Mean±SEM; *** Significant at P<0.001; ** Significant at P<0.01; * Significant at P<0.05 compared to glimepiride control

Figure No 1: Glimepiride (0.41 mg/kg) declined % blood glucose levels in healthy albino rats earlier and later treatment with bosentan (10 mg/kg).



Impact of Bosentan Pre-treatment on Glimepiride's anti-diabetic action in diabetic rats: During this study, pre-treatment of bosentan (10 mg/kg for seven days) significantly affected the onset of hypoglycemia from 1 hour i.e. 18.03 ± 1.23 % reduction before treatment to 2 hours i.e. 16.03 ± 0.48 % reduction after treatment. However, peak hypoglycemia was affected significantly i.e. 48.49 ± 2.09 % reduction before treatment to 34.10 ± 0.42 % reduction after treatment at the 8th hour. The duration of hypoglycemia was 24 hours and reduced to 18 hours only after treatment (17.89 ± 1.32% reduction before treatment and 16.46 ± 0.38 % reduction after treatment). Table no 2 shows the results, whereas Figure 2 shows them graphically.

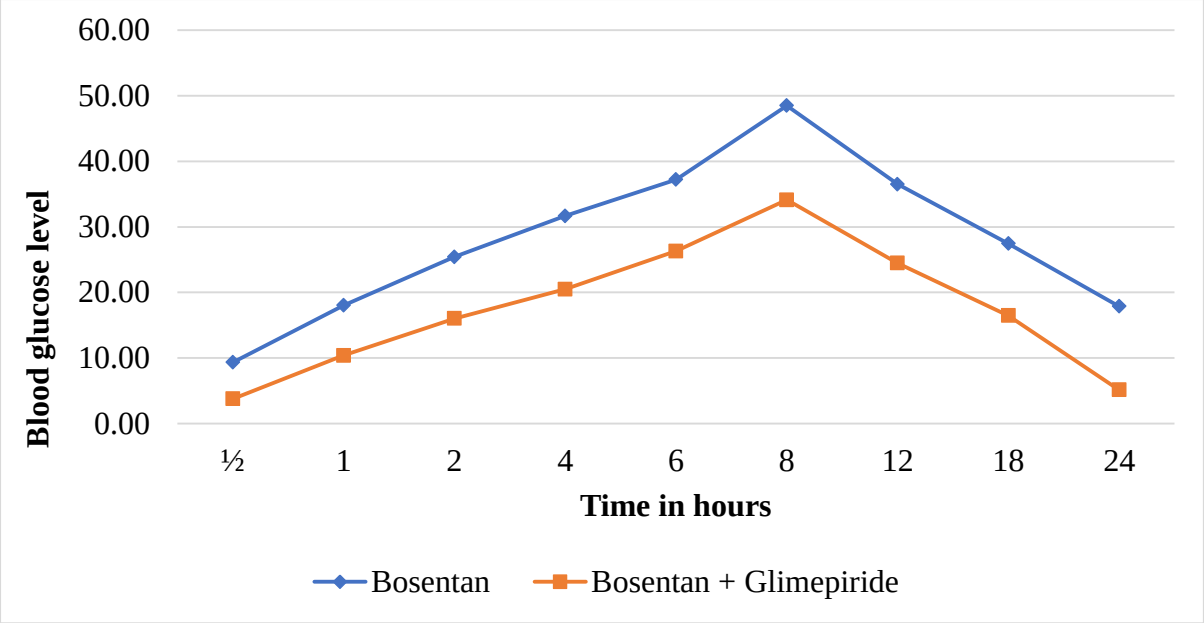
Table No. 2: The percentage levels of blood glucose in diabetic albino rats earlier and later therapy of bosentan (10 mg/kg) on glimepiride (0.41 mg/kg).

Time in Hrs	Glimepiride blood glucose levels in percentage (mg/dl)	Bosentan blood glucose levels in percentage (mg/dl)	Glimepiride + Bosentan blood glucose levels in percentage (mg/dl)
	Mean ± SEM	Mean ± SEM **	Mean ± SEM *
0	-	-	-
½	9.34±0.86	2.50±0.11	3.77±0.21***
1	18.03±1.23	6.49±0.13	10.38±0.44***
2	25.42±1.65	8.94±0.12	16.03±0.48***
4	31.67±1.74	11.53±0.08	20.50±0.54***
6	37.21±1.71	13.25±0.25	20.30±0.35***
8	48.49±2.09	8.48±0.14	34.10±0.42***
12	36.51±1.59	5.41±0.11	24.48±0.52***

18	27.46±1.48	2.36±0.09	16.46±0.38***
24	17.89±1.32	1.07±0.16	5.14±0.41***

Mean±SEM; *** Significant at P<0.001; ** Significant at P<0.01; * Significant at P<0.05 compared to glimepiride control

Figure No 2: Glimepiride (0.41 mg/kg) declined % blood glucose levels in diabetic albino rats earlier and later treatment with Bosentan (10 mg/kg).



Discussion:

Patients with diabetes mellitus are at a higher risk of developing hypertension. In such circumstances, an antidiabetic medicine like glimepiride, as well as antihypertensive agents, must be given at the same time. According to a literature survey, there are many drug interactions are found such as rifampicin has been reported to stimulate rosiglitazone metabolism [21] and pharmacokinetic interaction between Tadalafil and bosentan in healthy male subjects [22]. So, Between the antihypertensive agent and the agents which are metabolised by these enzymes, there's a chance of a drug-drug interaction. Glimepiride is primarily metabolized in the liver by CYP2C9 to the active M_1 (hydroxyl) metabolite and then to the inactive M_2 (carboxy) metabolite. [11]. In our experiment, rats showed that when Bosentan and oral antidiabetic medications are administered together in healthy animals, there is a drug-drug interaction. However, the connection in pathophysiological situations such as diabetes was unclear. As a result, in our experiment, Alloxan-induced diabetic rats were adopted, The diabetic rats were administered glimepiride orally, and the onset, peak and duration of hypoglycemia were measured. The same animals were got pretreatment for one week with bosentan (10mg/kg), followed by glimepiride. Bosentan markedly change the onset of hypoglycemia of glimepiride i.e. (18.03±1.23% reduction before treatment at 1 hour to 16.03±0.48% reduction after treatment at 2 hours) also it did markedly reduce the peak hypoglycemia (48.49±2.09% reduction before treatment to 34.10±0.42% reduction after treatment) at 8^{th} hour. Bosentan reduces the duration of hypoglycemia of glimepiride i.e. from 24 to less than 18 hours (17.89±1.32% reduction before treatment to 16.46±0.38% reduction after treatment).

Conclusion:

In both healthy and diabetic rats, a single dose of bosentan did not affect blood glucose levels. These findings suggest that bosentan has no hypoglycemic effect, implying that the drug-drug interaction with glimepiride is of the pharmacokinetic type. Bosentan pre-treatment for one week affected the onset, duration and decrease peak hypoglycemia produced by glimepiride in both healthy and diabetic rats.

The induction of the CYP2C9 isoenzyme systems by bosentan may be responsible for the decrease in the hypoglycemic action of oral antidiabetic drugs. As a result of the foregoing conclusion, it is reasonable to conclude that blood glucose monitoring is required when glimepiride and bosentan are used together. When oral antidiabetic medicines are used concurrently with bosentan, it is also recommended that the dose and dosing frequency should be adjusted.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

References:

1. H. Sheth, D.K. Suresh, R. Hasan, Md S. Khalid and S. Mahesh, 2011. Influence of Bosentan on Antidiabetic Effect of Pioglitazone and Nateglinide in Experimental Animals. *Journal of Pharmacology and Toxicology, 6: 427-432.*

2. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, *et al*. Impaired fasting glucose and impaired glucose tolerance: Implications for care. Diabetes Care 2007;30:753-9.

3. Rehman A, Setter SM, Vue MA. Drug-induced glucose alterations Part 2: Drug-induced hyperglycemia. Diabetes Spectr 2011;24:234-8.

4. Hasnain H, Ali H, Zafar F, Akbar AS, Hameed K, Shareef H, *et al*. Drug-drug interaction; facts and comparisons with national and international bentch marks; a threat more than a challenge for patient safety in clinical and economic scenario. Prof Med J 2017;24:357-65.

5. Martins IJ. Drug-drug interactions with relevance to drug induced mitochondrial toxicity and accelerated global chronic diseases. ECPT 2017;3:18-21.

6. Clement S, Braithwaite SS, Magee MF, Ahmann A, Smith EP, Schafer RG, *et al*. Management of diabetes and hyperglycemia in hospitals. Diabetes Care 2004;27:553-91.]

7. Tatro DS. Drug Interaction Facts 2004. 1st ed. New York; Facts & Comparisons; 2003.

8. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Phys Ther 2008;88:1322-35.

9. Hammad MA, Mohamed Noor DA, Syed Sulaiman SA, Aziz NA, Elsobky Y. A prospective study of prevalence of uncontrolled glycaemia in type 2 diabetes mellitus outpatients, 2016 ACCP Virtual Poster Symposium. Pharmacotherapy 2016; 36:e83-138.

10. Srinivas NR. Clinical drug-drug interactions of bosentan, a potent endothelial receptor antagonist, with various drugs: Physiological role of enzymes and transporters. Gen Physiol Biophys. 2016 Jul;35(3):243-58. DOI: 10.4149/gpb_2015050. Epub 2016 Apr 5. PMID: 27045668.

11. Basit, A., Riaz, M., & Fawwad, A. (2012). Glimepiride: evidence-based facts, trends, and observations (GIFTS). [corrected]. *Vascular health and risk management, 8*, 463–472. https://doi.org/10.2147/HIV.S33194.

12. Niemi, M., Kivistö, K. T., Backman, J. T., & Neuvonen, P. J. (2000). Effect of rifampicin on the pharmacokinetics and pharmacodynamics of glimepiride. British journal of clinical pharmacology, 50(6), 591-595.

13. Prashant Suresh Salunke, Arindam Das, D.K. Suresh, Jyotsna Pandit Khedkar, Influence of Bosentan on Pharmacokinetics of Pioglitazone and Nateglinide on Experimental animal, Adv. Biores., Vol 12 (3) May 2021: 93-101.

14. Mohammad AbdusSalam, Mohammad AbdullahilBaki, ZafrulAzamATM, Md. Shah Amran, Farhad Mohammad Amjad. (2009). In vitro and in vivo effects of glipizide and gliclazide on the protein binding, plasma concentration and serum glucose, cholesterol and creatinine levels of ibuprofen. Journal of pharmacology and toxicology 11. 23-28.

15. Mohammad Mohiuddin, ZafrulAzam ATM, Md. Shah Amran, Md.Amjad Hossain. (2009). In vivo effects of Gliclazide and metformin on the plasma concentration of caffeine in healthy rats. Pakistan Journal of Biological Sciences; 12(9): 734-737.

16. Kayamkani Abedulla Khan, Sreemantula Satyanarayana , Kilari Eswar Kumar,(2008). The mechanism of drug interactions of a selected antiarrhythmic drug with metformin, in different animal models", Brazilian Journal of Pharmaceutical Sciences DOI: http://dx.doi.org/10.1590/s2175-97902017000400054 A.

17. Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann. Clin. Biochem.6:24-27.

18. Sherie Pankaj Sharma, Ashish Prakash Anjankar, Anita Kale, Comparison of glucose levels using glucometer and GOD-POD Method in diabetic patients, International Journal of Clinical Biochemistry and Research 2017;4(1):6-10.

19. Shannon Reagan-Shaw, MinakshiNihal, Nihal Ahmad. (2007). Dose translation from animal to human studies revisited. Conversion of animal doses to HED based on BSA (Table-1). The FASEB Journal; 22:660.

20. Kayamkani Abedulla Khan, Sreemantula Satyanarayana, Kilari Eswar Kumar, (2017) The mechanism of drug interactions of a selected antiarrhythmic drug with metformin, in different animal models", Brazilian Journal of Pharmaceutical Sciences Braz. J. Pharm. Sci. vol.53 no.4 São Paulo 2017 Epub Jan 08, 2018 DOI: http://dx.doi.org/10.1590/s2175-97902017000400054 A.

21. Ji-Young Park [1], Kyoung-Ah Kim, Mun-Ho Kang, Su-Lyun Kim, Jae-Gook Shin (2004) Effect of rifampin on the pharmacokinetics of rosiglitazone in healthy subjects. Clinical pharmacology and therapeutics 2004 Mar;75(3):157-62. DOI: 10.1016/j.clpt.2003.10.003.

22. DingemanseJ,YuA,Darstein C,Phillips DL,Mitchell MI. (2008). Pharmacokinetic interaction between Tadalafil and bosentan in healthy male subjects. J Clin Pharmacology;48(5):610-8.