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ANTI-LEISHMANIASIS, ANTI-UREASE, ANTI-BACTERIAL AND ANTI-OXIDANT ACTIVITIES OF *B*-HYDROXY KETO COMPOUNDS SYNTHESIZED THROUGH ALDOL REACTION

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Abstract
Aldol and its derivatives play crucial role in the field of biological research. Recently, Numerous pharmacological investigations on aldol and its compounds have been carried out. However, more research is needed to determine the significance of biological compounds. "The *β*-hydroxy carbonyl compound is the central core for different necessity of biological compounds. The *β*-hydroxy carbonyl compounds are synthesized by an aldol reaction of Cyclohexanone with 2- bromo, 3-bromo, 4-bromo benzaldehydes in the presence of a catalyst **(2-picolylamine)** and solvent (water & ethanol) gives a compound. Different biological studies were carried out of the synthesized compound, such as anti- bacterial, anti-fungal, anti-leishmaniasis, anti-urease. Among different substituted compounds some were found with few folds' better potentials than standard drug E. coli, Terbinafine, thiourea and paromomycin.

Key Words: Synthesis, Aldol, Anti-urease, Anti- fungal, Anti-bacterial, anti-leishmaniasi.

Introduction
One of the most crucial areas of organic chemistry is organic synthesis (1). Carbon-Carbon bond is important aspects of modern organic synthesis, and one of the effective methods used for the formation of C-C bond is aldol reaction, where enolates of an aldehyde or ketone added to a carbonyl part at the *α*-carbon under basic or acidic conditions to obtain *β*-hydroxy aldehyde or ketone called

β-hydroxy carbonyl compound, which is primary products of aldol reaction (2). When aldehydes or ketones serve as carbonyl acceptors, a new stereo-center on the *β*-carbon atom is introduced, and the synthesis of *β*-hydroxy carbonyl compounds is accomplished by manipulating the absolute configuration of the *β*-carbon atom. (3). The direct catalytic aldol reaction is a new catalytic approach that allows atom-cost-effective utilization of functionalized chiral building blocks. (4). Chiral *β*-hydroxy carbonyl compounds, which serve as the basis antibiotics such as pheromones (5), and many biologically active compounds like anti-diabetic (6), anti-depressant (3), anti-oxidant (7), anti-analgesic (8), anti-inflammatory (9), anti-malarial (10), anti-cancer (11), anti-microbial (12).
The literature study shows that aldol reaction and their primary product *β*-hydroxy carbonyl compound are impressive pharmacophores with outstanding biological activity. Impressed by these observations, we synthesized a number of reported *β*-hydroxy carbonyl compound derivatives looking for more effective biological activity agents (such as anti-bacterial, anti-leishmaniases, anti-fungal, anti-urease agents). The synthesized compounds have been tested for biological activities.

Experimental

General procedure

The chemicals used in this work were analytical grade and purchase from Sigma Aldrich (Germany). Glass wares for reactions and separation were used in the work made up of Pyrex and borosilicate. Thin layer chromatography (TLC) was conducted using pre-coated silica gel plates (Kieselgel-60 HF 254; Merck, Germany) and spots were visualized through a UV lamp emitting dual wavelengths at 254 and 365 nm.

Synthesis of aldol derivatives

At ambient temperature, a mixture of cyclohexanone and a catalyst (2-picolyl amine: Acetic Acid, 20 mol%) was stirred in the presence of a solvent (1:1 ratio of 5 ml water to 5 ml ethanol).
After a period of time, bromo constituent aldehydes were dropped wise added to the mixture. The reaction progress was observed using Thin Layer Chromatography (TLC) with a solvent mixture of n-Hexane and Ethyl Acetate in a ratio of 2:8. Compounds formed two layers and were separated via separating funnel using $CHCl_3$ as solvent, an excellent yield was produced with high purity.

Synthesis of 2-((2-bromophenyl) (hydroxy)methyl)cyclohexanone

^{1}H-NMR (400 MHz, CDCl3) δ = 7.52–7.56 (m, 1H, H-12, Ar-H), 7.34–7.38 (m, 1H, H-13, Ar-H), 7.13–7.17 (m, 1H, H-14 Ar-H), 5.32 (d, J = 8.0 Hz, 1H, H-15, Ar-H), 2.68–2.71 (m, 1H, H-8, -CH), 2.35–2.38 (m, 1H, H-2, -CH), 2.07–2.10 (m, 2H, H-6, -CH_2), 1.83–1.86 (m, 2H, H-3 -CH_2), 1.69–1.72 (m, 2H, H-5, -CH_2), 1.58–1.68 (m, 2H, H-4 CH_2). (13).

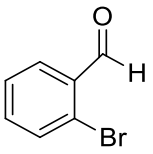
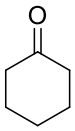
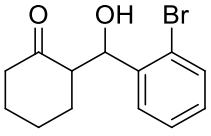
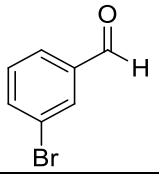
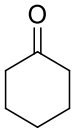
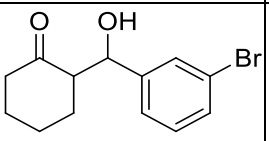
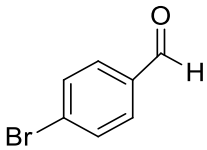
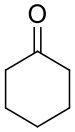
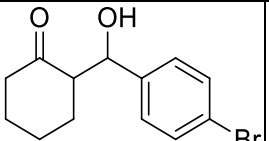
Synthesis of 2-((3-bromophenyl)(hydroxy)methyl)cyclohexanone

syn diastereomer, **^{1}H NMR (400 MHz, CDCl3)** δ = 7.48 (s, 1H, H-15, Ar-H), 7.37 (m, 1H, H-13, Ar-H), 7.21 (m, 2H, H-11,12, Ar-H), 5.35 (s, 1H, OH), 3.10 (d, J ¼ 4 Hz, 1H, H-8, -CH), 2.52–2.64 (m, 1H, H-5 -CH_2), 2.32–2.49 (m, 2H, H-1, -CH_2), 2.04–2.14 (m, 2H, H-4, -CH_2), 1.80–1.90 (m, 2H, H-2, -CH), 1.51–1.75 (m, 2H, H-3, -CH_2); anti diastereomer, **^{1}H NMR (400 MHz, CDCl3)** δ = 7.42 (m, 1H H-11, Ar-H), 7.49 (m, 1H, H-13, Ar-H), 7.22 (m, 2H, H-14,15 Ar-H), 4.74 (d, J ¼ 8 Hz, 1H, H-8, -CH), 4.02 (s, 1H, -OH), 2.52–2.62 (m, 1H, H-2, -CH), 2.42–2.51 (m, 2H, H-6, -CH_2), 2.29–2.40 (m, 2H, H-3, -CH_2), 1.76–1.84 (m, 2H, H-5, - CH_2) 1.52–1.74 (m, 2H, H-4, - CH_2) (14)

Synthesis of 2-((4-bromophenyl)(hydroxy)methyl)cyclohexanone

(mix, syn/anti 50: 50): anti-diastereomer,**^{1}H NMR (400 MHz, CDCl3)** δ = 7.46 (m, 2H, H-12,14, Ar-H), 7.19 (m, 2H, H-11,15, Ar-H), 4.75 (dd, J ¼ 8 Hz, 0.42 1H, H-8, -CH), 3.99 (s, 0.41H OH), 2.51–2.90 (m, 1H H-2, -CH), 2.42–2.50 (m, 2H, H-5, -CH_2), 2.30–2.41 (m, 2H, H-3, -CH_2), 2.04–1.13 (m, 2H, H-5, -CH_2), 1.75–1.88 (m, 2H, H-4, -CH_2);syn diastereomer, 5.33 (s, 0.56H) 3.08 (s, 0.53H) (14)

Table-1: Represent substituted compounds along with yield and time

Reactant no 1	Reactant no 2	Products	Yield %	Time T (hour)
		 **SRL1**	92% obtained 89% (15) 86% (14).	5 h completed (16 h) (15) (75 h) (13)
		 **SRL2**	80% obtained 72% (13)	38 h completed (75 h) (13)
		 **SRL3**	85% obtained (89% (13) 75% (14).	7 h completed (68 h) (13) (16h) (14).

Catalyst: 2-picolylamine: A. Acid 20mol%)
Solvent: 5ml Water:5ml Ethanol 1:1

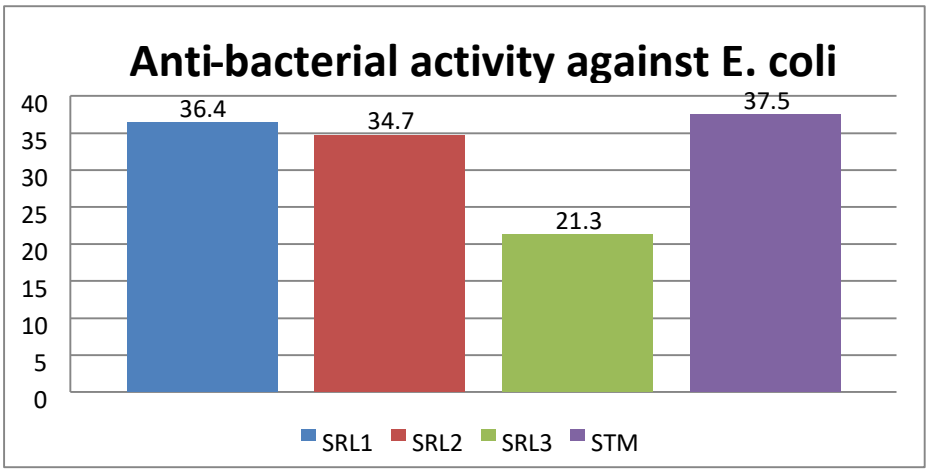
Results

Biological profile

After completions of synthesis, the compounds were tested for biological activity for the purpose to evaluate its efficacy. In this regard, anti-bacterial, anti-fungal, anti-urease, and anti-leishmanial activities were carried out in the presence of their standard drugs, including Streptomycin (37.5%), Terbinafine (50.7%) Thiourea (IC_{50} = 15.10 ± 0.40 μM) and Paromomycin (IC_{50} = 10.10 ± 0.30) respectively.

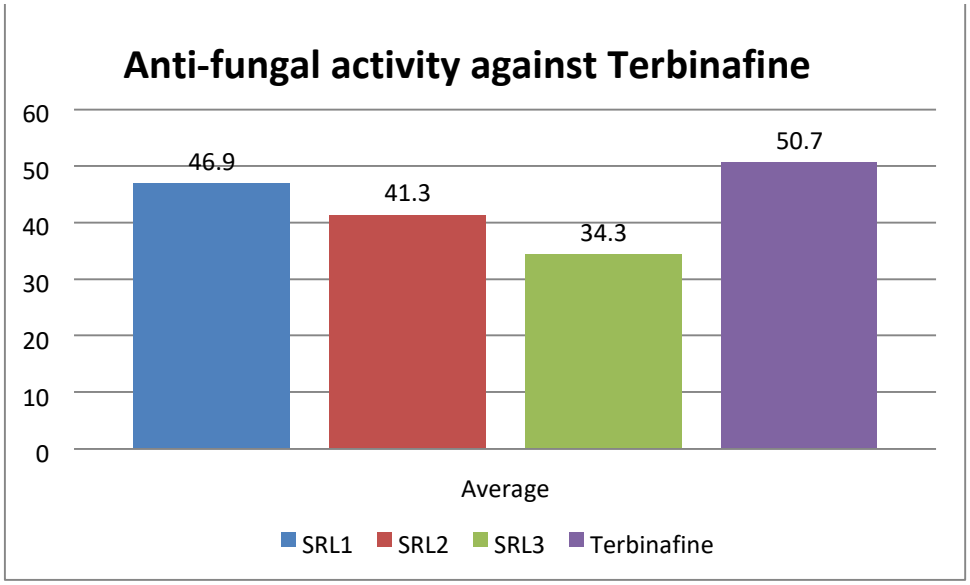
Anti-bacterial activity

The anti-bacterial activity was carried out for all compounds, showed different percentage of inhibitions might be the presence of varied position of bromine.
Compound **SRL1**(36.4%) bearing bromo moiety at *Ortho*-position displayed excellent activity.
Compound-**SRL2** (34.7%)**,** exhibited much better result than **SRL3,** the inhibition might be the attachment of bromo group at *meta*-position of aromatic ring, thus attractive result was found.
Compound-**SRL3** bearing bromo moiety at *para*-position, displayed 21.3% inhibition when relate to standard drug (37.5%).



Anti-fungal activity

The results of anti-fungal investigation revealed that compound **SRL1** compound indicate the most promising activity against Terbinafine. Compound **SRL2** exhibited outstanding efficacy, and **SRL3** show average activity against standard drug.



Anti-urease activity

To determine the most effective molecules, all of the synthesized compounds were evaluated for anti-urease activity. The outstanding activity indicated by meta-substituted molecule may be due to enhanced interaction by meta-site, while para and orthro-substituted moieties were ranked second and third, respectively.

S/No	**Compounds**	**IC_{50} anti-Urease (µM ± SEM)**
1	SRL1	12.18 ± 0.10
2	SRL2	14.20 ± 0.30
3	SRL3	21.10 ± 0.20
Stan	**dard drug Thiourea**	**15.10 ± 0.40**

Anti-leishmanial activity

The anti-leishmanial activity was carried out for all three compounds against standard drug paromomycin. The compound **SRL2** and **SRL1** shows excellent potential results against standard drug. Compound **SRL3** also indicated attractive result.

S/No	**Compounds**	**IC_{50} anti-leishmanial (µM ± SEM)**
1	SRL1	7.20 ± 0.20
2	SRL2	11.10 ± 0.50
3	SRL3	17.20 ± 0.10
Standard drug Paromomycin		**10.10 ± 0.30**

Conclusion

In present study we studied the reported synthesized bromo substituent primary aldol moieties. The novelty of reaction is catalyst **(2-picolylamine)** used in this reaction is not reported in the literature for such aldol adduct. The catalyst worked amazingly and shows good result for the reaction, and

shows better yield when relate with reported worked, Also, the time was enhanced as reported in literature. Different activities such as anti-bacterial, anti-fungal, anti-urease and anti- leishmanial activities was screened out for the compounds, which shows very impressive results against standard drugs. Compound **SRL1** (39.2%) and **SRL2** (34.6%), when compared to the standard Drug E. coli (37.5%), showed strong inhibitory activity when the potentials were tested against bacterial strains. Similarly, the anti-fungal inhibitory activity study of compound was carried out against standard drug terbinafine (50.7%), in which compound **SRL1** (46.9%), **SRL2** (41.3%) shows effective results.

The potential of Bromo substituted compounds **SRL2** (14.20 ± 0.30 μM) and **SRL1** (12.18 ± 0.10 μM) was found to be authentic than that of the standard drug Thiourea (IC_{50} = 15.10 ± 0.40 μM). Moreover, the compounds were also tested against anti-leishmanial standard drug Paromomycin (IC_{50} = 10.10 ± 0.30 μM), compound **SRL1** (7.20 ± 0.20 μM) and **SRL2** (11.10 ± 0.50 μM) was found to very active against the standard drug.

Future prospective

In the future the compound SRL1 could a better drug because it shows strong inhibitory activity when relate with standard drug E. coli and Terbinafine, and if SRL2 compare with standard Thiourea and Paromomycin, it can be more effective moiety against standard drugs.

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References

1. Wiebe A, Gieshoff T, Möhle S, Rodrigo E, Zirbes M, Waldvogel SRJACIE. Electrifying organic synthesis. angew chem. 2018;57(20):5594-619.
2. Zijian L, Toshifumi T, Roman P, Fernando AA, Naoya K, Masakatsu S. Direct Catalytic Asymmetric Aldol Reaction of α-Alkylamides. Org. Lett. 2017;19(3):710-713.
3. Shimizu Y, Shi SL, Usuda H, Kanai M, Shibasaki MJACIE. Catalytic Asymmetric Total Synthesis of ent-Hyperforin. Angew. Chem. 2010;49(6):1103-6.
4. Kühbeck D, Bachl J, Schön EM, Gotor-Fernández V, Díaz Díaz DJHCA. Gelatin Protein-Mediated Direct Aldol Reaction. Hel. Chim. Acta. 2014;97(4):574-80.
5. Dibello E, Seoane G, Gamenara DJSC. Green and Catalytic Synthesis of Dominicalure I, Major Component of the Aggregation Pheromone of Rhyzopertha dominica (Fabricius)(Coleoptera: Bostrichidae). Synth. Commun. 2015;45(8):975-81.
6. Hsieh C-T, Hsieh T-J, El-Shazly M, Chuang D-W, Tsai Y-H, Yen C-T, et al. Synthesis of chalcone derivatives as potential anti-diabetic agents. Bioorg. Med. Chem. Lett. 2012;22(12):3912-5 .
7. Kaur P, Das P, Chaudhary A, Singh BJNPC. Naturally Occurring Limonene to Cinnamyl-type γ-Butyrolactone Substituted Aldol Condensation Derivatives as Antioxidant Compounds. Nat. Prod. Commun. 2012;7(9):1934578X1200700903.
8. Li W, Li X, Wu W, Liang X, Ye JJCC. Highly diastereo-and enantioselective one-pot Michael–Aldol reactions of α, β-unsaturated aldehydes with imidazole derivatives. Chem. Commun. 2011;47(29):8325-8327.
9. Dewu Z, Hanlin G, Jian-hua Z, Xiaoyu T, Ridao C, Jungui D. Periconianone A, a New 6/6/6 Carbocyclic Sesquiterpenoid from Endophytic Fungus Periconia sp. with Neural Anti-inflammatory Activity. Org. Lett. 2014;16(5):1410–1413.
10. Eagon S, Hammill JT, Fitzsimmons K, Sienko N, Nguyen B, Law J, et al. Antimalarial activity of 2, 6-dibenzylidenecyclohexanone derivatives. Bioorg. Med. Chem. *Lett.* 2021;47:128216.
11. Yang J, Mu W-W, Liu G-YJEJoP. Synthesis and evaluation of the anticancer activity of bischalcone analogs in human lung carcinoma (A549) cell line. Eur. J. Pharmacol. 2020;888:173396.
12. Boateng A, Amano M, Sugiura MJAo. Synthesis of Selenoesters via Aldol Condensation and/or

Conjugate Reduction and Their Antiviral Activities. J. Am. Chem. Soc. 2022;8(1):1369-74.
13. Zhang F, Li C, Qi CJTA. Highly diastereo-and enantioselective direct aldol reaction under solvent-free conditions. Tetrahedron: Asymmetry. 2013;24(7):380-388.
14. Li L, Fang Z, Fang J, Zhou J, Xiang YJRa. d-Fructose-derived β-amino alcohol catalyzed direct asymmetric aldol reaction in the presence of p-nitrophenol. RSC Adv. 2013;3(43):21084-91.
15. Chen F, Huang S, Zhang H, Liu F, Peng YJT. Proline-based dipeptides with two amide units as organocatalyst for the asymmetric aldol reaction of cyclohexanone with aldehydes. Tetrahedron lett. 2008;64(40):9585-91.