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Chemical Separation of Compounds from Antituberculosis Drug and Their Antibacterial Biological Activity

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ABSTRACT

Background and aims. *In this study separated between the active ingredients of the expired isoniazid. The separated substance may enhance antibacterial biological activity.* ***Methods.*** *The target compounds were designed is added to the ketone (acetyl acetone) as the primary amine (isoniazid), the other to the preferred substance (isoniazid) with the aldehyde (benzaldehyde), and the final reaction is the separated substance (isoniazid) with (p-methoxy benzaldehyde) were evaluated for antibacterial biological activity against tested clinical Escherichia coli (E. coli) and Methicillin Resistant Staphylococcus aureus (MRSA)and were subjected to molecular properties and bioactivity prediction by the Molinspiration.* ***Results.*** *The compound (1-isonicotinoyl-3,5-dimethyl-4-H-pyrazol-1-ium) exhibited significant antibacterial activity on tested gram negative and gram-positive bacteria. Furthermore, compounds (E)-N-benzylidene isonicotiolryazide) and (E)-N'-(4-methoxybenzylidene) isonicotinohydrazide showed intermediate inhibitory activity against gram-negative Escherichia coli (E. coli). Furthermore, all compounds obeyed Lipinski's rule of five. Also, compounds (E)-N-benzylidene isonicotiolryazide) and (E)-N'-(4-methoxybenzylidene) isonicotinohydrazide have drug-like properties within those considered adequate for a drug candidate.* ***Conclusion.*** *separating expired drugs has a helpful effect in discovering drugs.*

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INTRODUCTION

The world has witnessed a great development in the pharmaceutical industry until the number of manufactured and unprocessed medicines reached 2019 between (580-334) drugs [1,2]. The presence of this huge number of manufactured varieties is the problem of getting rid of expired medicines due to the effect of these problems on the environment. Most of the problems with these expired drugs come from the fact that they stay a long time in the ground when they are thrown away without decomposing. It led to major environmental problems affecting plants, animals, soils, and groundwater, and thus reaching man in one way or another, causing him many serious diseases. Many studies have focused on getting rid of expired medicines, whether by burning, landfilling or other methods. The rise in population growth necessarily leads to more waste of different products. The term waste recycling has gained global consideration as a perfect solution to reduce the waste problem. Separation and reusing the raw materials are considered a great technique to benefit from the waste. Drugs are one of these commonly used products, which frequently end up in the trash, in bathrooms, or burned) [3, 4]. This waste has special characteristics that differ from others, as most of them do not decompose when thrown into the soil, which leads to pollution in groundwater and plants that reach humans later**.** [5, 12]**.** Even worse, the emergence of resistant bacteria because of the presence of antibiotics in surface and groundwater [13]**.** A number of attempts to overcome drug wasting have been established. Despite this, there are no serious attempts to reuse the active ingredients contained within these medicines. In addition, some expired medicines still retain their high efficacy [14,15]. The drug usually contains an active ingredient and additives, which are considered the most important components inside. They cause a greater impact on the environment than the rest of the additives.

Separating the active ingredients enables scientists to obtain them in pure forms, which helps to reuse them in several fields as important organic substances with a distinctive composition. In addition, they are economically more attractive than synthesizing them from scratch. An earlier study in drug recycling was accomplished by recovering an undisclosed API from tablets with recognized excipient contents and compositions by solid−liquid extraction, membrane separation, and antisolvent crystallization [16]. Unfortunately, the possibility of using this process to recover other types of APIs was not being investigated. Although the toxic and environmental impact of medicines is less than that caused by pesticides, it has been shown that some types of medicines, such as parasite resistance, antifungals, and toxins, have an effective negative impact on the environment [17]. Although the toxic and environmental impact of medicines is less than that caused by pesticides, it has been shown that some types of medicines, such as parasite resistance, antifungals, and toxins, have an effective negative impact on the environment. The decline of vulture populations on the Indian subcontinent because of harming with Diclofenac, a non-steroidal pain reliever, is a genuine illustration of how startling openness pathways can prompt extreme ecotoxicological impacts. Anti-mycotic and anti-cancer medicinal products are drug groups that are particularly expected to kill their objective life form or target cells and might end up being the main drug intensifiers influencing human wellbeing through environmental exposure. Persistent low-level exposure to therapeutic items can happen through drinking water and through deposits in leaf crops and root crops [18]. Furthermore, experiment with the different techniques to separate active pharmaceutical ingredients from the other excipients. Reusing the active pharmaceutical ingredients in known chemical reactions is essential for the biological properties of the new derivatives.
Separate compounds from INH are added to ketone (acetyl acetone) or aldehyde (benzaldehyde), and the structural features of the biological antibacterial properties of design compounds are studied.

METHODS

Chemistry

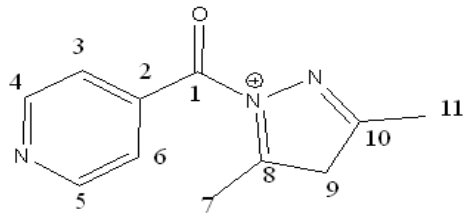
The extraction method was first tested using a mixture of water and ether solvent. Unfortunately, this technique did not give the required results due to the solubility of the active substance in water. The second method that has been tried is to exploit the difference in the densities of the isoniazid and the excipients by using centrifuges. This method gave a better result.

Table 1. Density of the mixture between Hexane and carbon tetrachloride)

N0	Hexane d = 0.659 g/mL	carbon tetrachloride d= 1.59g/ml	Density of the mixture
1	0	10	15.74=1.574
2	1	9	14.58=1.458
3	2	8	13.63=1.363
4	3	7	12.73=1.273
5	4	6	11.85=1.185
6	5	5	10.9=1.09
7	6	4	10.15=1.015
8	7	3	9.14=0.914
9	8	2	8.34=0.834
10	9	1	7.39=0.739

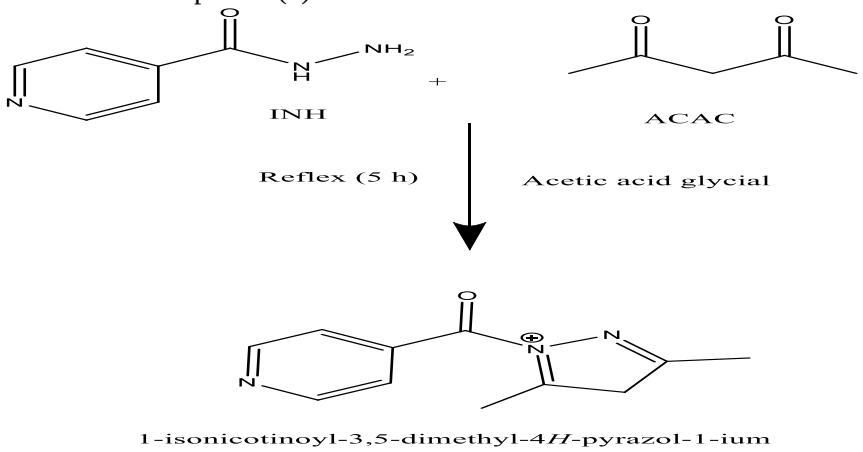
As mentioned in table (1), in this method, different values of the two solvents were mixed in order to obtain different density values for the mixture. The density of the two solvents combined should be higher than the density of the excipients but less than the density of the active substance. When this mixture is placed in a centrifuge, we can separate the active substance from the excipients. Since the density of isoniazid is 1.2 g/cm3 and the density of the excipients varies between zero and 1.09 g/cm3), The best mixture is number 6 from the table where the value of the density of the mixture is (1.09 g/cm3). It is lower than the density of isoniazid and higher than the density of the additives.

Synthesis of compound 1



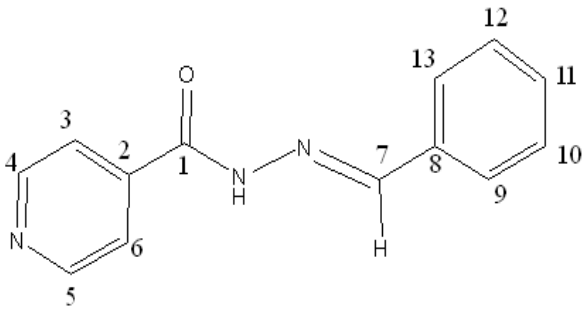
1-isonicotinoyl-3,5-dimethyl-4 *H*-pyrazol-1-ium

A mixture of (INH) (1.5 g) and (ACAC) acetyl acetone (1.1 ml) in glacial acetic acid (15 mL) is refluxed for 4-5 hours. The resulting mixture was concentrated and allowed to cool. The resulting solid was filtered, washed, dried, and recrystallized from ethanol to afford compound (1).



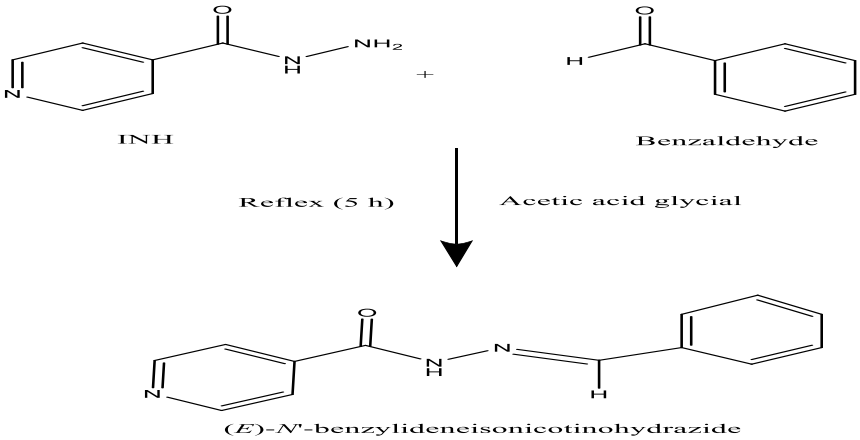
1-isonicotinoyl-3,5-dimethyl-4*H*-pyrazol-1-ium

Synthesis of compound 2



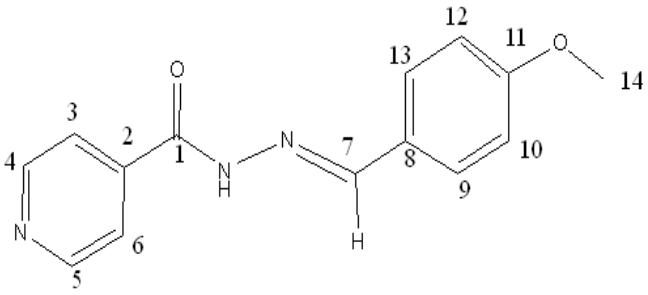
(*E*)-*N*'-benzylideneisonicotinohydrazide

A mixture of (INH) (1.5 g) and benzaldehyde (2.2 ml) in glacial acetic acid (15 mL) is refluxed for 4-5 hours. The resulting mixture was concentrated and allowed to cool. The resulting solid was filtered, washed, dried, and recrystallized from ethanol to afford compound (2).



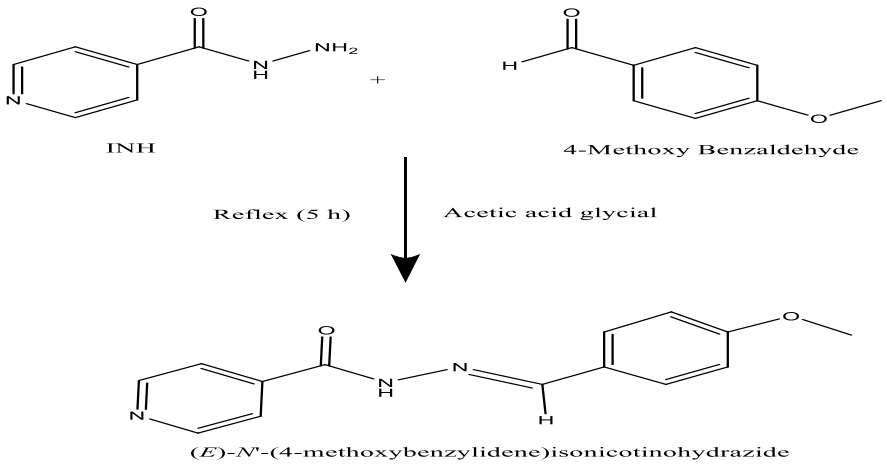
(*E*)-*N*'-benzylideneisonicotinohydrazide

Synthesis of compound 3



(*E*)-*N*'-(4-methoxybenzylidene)isonicotinohydrazide

A mixture of (INH) (1.5 g) and 4-methoxy benzaldehyde (1.3 ml) in glacial acetic acid (15 mL) is refluxed for 4-5 hours. The resulting mixture was concentrated and allowed to cool. The resulting solid was filtered, washed, dried, and recrystallized from ethanol to afford compound (3).



(*E*)-*N*'-(4-methoxybenzylidene)isonicotinohydrazide

Biological Activity

The disc diffusion method was carried out according to Abdulla-Eltawaty et al., (2021) with some modifications to investigate the antibacterial biological activity against tested clinical Escherichia coli (E. coli) and Methicillin Resistant Staphylococcus aureus (MRSA). For each of the three tested compounds, triplicate sterilized filter paper discs (Whatman

No. 1, 6 mm in diameter), freshly socked with 20 µl of the tested compound, were placed on the surface of a Muller Hinton Agar plate. The agar plates were already inoculated with 100 l of freshly prepared bacterial suspension (adjusted with 0.5 MacFaralnd standard). The agar plates were incubated at 37°C for 18 hours. With the use of a ruler, the diameters and means of inhibition zones were measured and calculated, respectively. The sensitivity of bacterial isolates to the plant essential oil was classified as not sensitive for a diameter less than 8 mm, moderately sensitive for a diameter range from 8 to 14 mm, sensitive for a 14–20 mm diameter, and very sensitive for a diameter larger than 20 mm [19].

Computational studies

In the present investigation, the active compounds 1, 2, and 3 were subjected to molecular properties and bioactivity prediction by the Molinspiration online property calculation toolkit [20], and ADME profiling by PreADMET [21] to filter and analyze their overall potential to qualify for a drug.

In the majority of cases, an orally available drug candidate is obedient to Lipinski's rule if lipophilicity (LogP) is no more than 5, molecular weight (MW) is less than 500, the number of hydrogen bond donors (HBD) is less than 5, and the number of hydrogen bond acceptors (HBA) is less than 10 [22]. submitted that great oral bioavailability in rats existed observed if a compound had 10 or fewer NROTB and TPSA of less than 90 Å2 [22]. Pharmacokinetic properties play an impart role in the development of successful drug. Pharmacokinetic properties including absorption, distribution, metabolism, excretion, and toxicity (ADMET) were calculated using PreADMET.

RESULTS AND DISCUSSION

Chemistry

Separation depends on the density difference between the active substance in the drug (INH) and the other substances. Separation was confirmed by melting point measurement and a TLC test. Schiff base is the separated substance is added as a primary amine (INH) with a ketone (ACAC) in the first reaction, and the second reaction is the addition of the preferred substance (INH) with an aldehyde (benzaldehyde). The third reaction is the separated substance (INH) with (pmethoxybenzaldehyde). Four analyses were performed on all the products, and the analyses are (IR, 1HNMR, 13CNMR, and Mass Spectra).

Compound 1 (1-isonicotinoyl-3,5-dimethyl-4-H-pyrazol-1-ium)

Yield: 85.9 %, M.P.:250 °C, M.W.: 202.24, IR (KBr, cm-1) 3425.58 (-NH),: 3101.54 (Ar-H), , 1705.07 (C=O), 1650-2000 (-CH), 1566.20 (C=N), 1411.89(C=C), 1149.57 (C-O),.H1 NMR: α 8.762 (s, 1H, Ar-H), 8.747 (s, 1H, Ar-H), 7.803 (s,1H, ArH) ,7.788 (s, 1H, Ar-H), 3.71 (s, 3H,- CH3), 3.38 (s, 3H,- CH3),2.43 (s, 2H). ; C13 NMR: α 171.195, 155.548 , 143.133, 127.774 , 44.963 , 43.861, 44.636 , 44.142 , 44.415; MS(m/z):80.75 (M+, 1 00), 202.38 (M+1, 29.04).

Compound 2 (E)-N-benzylidene isonicotiolryazide)

Yield :89%, M.P.:192 Co, M.W.: 226, IR (KBr, cm-1) 3425.58 (-NH), 3194.12 (Ar-H), , 1689.64 (C=O), 1600-2000 (-CH), 1566.20 (C=N), 1411.89(C=C), 1149.57 (C-O),. H1 NMR: α 12.0 73(s, -NH) , 8.795 (s, 1H,Ar-H) , 8.780 (s, 1H), 8.125 (s, 1H ,Ar-H), 8.117(s, 1H), 7.841(s, 1H ,Ar-H), 7, 830 (s, 1H ,Ar-H), 7.751 (s, 1H ,Ar-H), 7. 747(s, 1H ,Ar-H), 7.672 (s, 1H ,Ar-H), 7.460 (s, 1H ,Ar-H),) , 7.435 (s, 1H ,Ar-H),7.398 (s, 1H ,Ar-H); : α 161.74,165.149 , 149.592 , 149.130 ,144.834, 140.509, 134.074, 130.417 , 130.030 , 128.899 ,127.328 , 126.926 ,123.223 ,121.606 40.320,40.054 , 39.781, 39.500 ,39.219 , 38.946 ,38.673;MS (m/z) : 78.11 (M+, 100), 226 (M+1, 2.65).

Compound 3 (E)-N'-(4-methoxybenzylidene) isonicotinohydrazide.

Yield: 93.1%, M.P.: 156 Co, M.W.: 255.10, IR (KBr, cm-1) 3441.01 (-NH), 3155.54 (Ar-H), 1658.78 (C=O), 1600-2000 (-CH), 1597.06 (C=N), 1411(C=C), 1411.89(C-O),1165 (COC). H1 NMR: α 11.921 (s, 1H ,-NH),8.783 (s, 1H ,Ar-H), 8.765(s, 1H ,Ar-H), 8.414(s, 1H), 7.825(s, 1H ,Ar-H), 7,806 (s, 1H ,Ar-H), 7.711(s, 1H ,Ar-H), 7.682 (s, 1H ,Ar-H), 7.039 (s, 1H ,Ar-H),,7.011(s, 1H ,Ar-H), 3.803 (s,1H), 3.762 (s,1H), 3.369 (s,1H) ;C13-NMR: α 161.590, 161.172 , 150.366, 149.585 ,149.107 ,140.684 ,129.034, 128.557 ,126.638 ,123.291,121.622 ,114.428 , 55.345, 40.062 .; MS (m/z) : 133 (M+, 100) , 255.10 (M+1, 1.82).

Biological Activity

Table2: Antibacterial Activity of Tested Compounds on Tested Gram Negative and Gram-Positive Bacteria

Name of Tested Sample	Mean of Diameter of Inhibition Zones (IZ) in Millimeter Against Tested Bacteria	
	Escherichia coli (E. coli)	Methicillin Resistant Staphylococcus aureus (MRSA)
Compound (1)	15 mm	18.5 mm
Compound (2)	11.5 mm	0 mm
Compound (3)	11 mm	7.5 mm

Inhibition zone (mm): (o-9 = weak), (10-12 = intermediate), (13-18 = active), (≥19= very active)

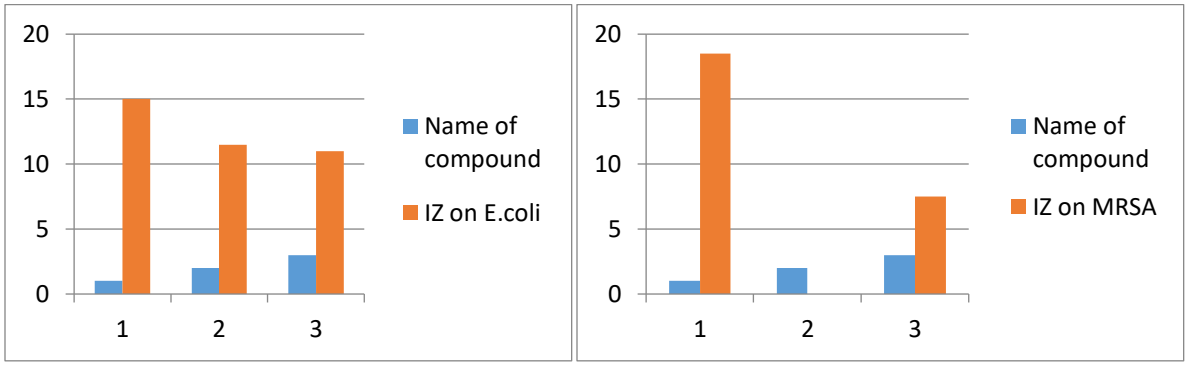


Figure 1. Antibacterial Activity of Tested Compounds on Tested Gram Negative and Gram Positive Bacteria

Computational studies

In *silico* prediction of physicochemical properties and pharmacokinetic profile Molecular property expectation is turning into a valuable apparatus in the age of particles with the right boundaries to be helpful medication applicants. Drug plan and lead advancement benefits from the capacity to anticipate actual properties like lipophilicity and dissolvability number of H-bond givers and acceptors to fabricate movement expectation apparatus which predicts drug similarity [22]. The estimated physicochemical properties of target compounds presented in Table 3.

These compounds were shown to retain significant for H-bond donors (0-1) and H-bond acceptors (3-4) as shown in table 1. Furthermore, for good membrane penetrability clogP value should be ≤ 5. Compounds indicated clogP -2.75 -1.87 value. The results exhibited that all compound conform Lipinski's rule of five. All tested compounds possessed (1-4) rotatable bonds. The results revealed that the compounds shown TPSA within suitable values (TPSA = 45.33-63.59**)**. The compounds shown to be low to medium cell permeability in the Caco-2 cell model with value 2.73- 21.15 nm/ sec (table 4). In addition, compounds exhibited medium absorption through MDCK. Besides, the tested compounds was expected to have weakly plasma protein binding (7.88-76.41 %).

The frontier compound revealed great HIA values (95.19- 98.01 %) indicating very well- intestinal absorbed compound . In addition, compounds exhibited low to medium CNS absorption.

Table 3: Calculated physicochemical properties of the frontier compounds

Cpds. ID	Molinspiration						
	cLogP	M.W	MF	HBA	HBD	TPSA	NROTB
1	-2.75	202.24	$C_{11}H_{12}N_3O$	4	0	45.33	1
2	1.81	225.09	$C_{13}H_{11}N_3O$	3	1	54.33	3
3	1.87	255.10	$C_{14}H_{13}N_3O_2$	4	1	63.59	4

LogP: logarithm of compound partition coefficient between n-octanol and water. **MW:** molecular weight **MF:** molecular Formula **HBA:** number of hydrogen bond acceptors. **HBD:** number of hydrogen bond donors. **TPSA:** topological polar surface area. **NROTB:** number of rotatable bonds.

Table 4: ADME data of tested compounds

Cpds. ID	PreADMET				
	Caco2[a]	MDCK[b]	HIA[c]	BBB[d]	PPB[e]
1	2.73	31.78	98.01	0.19	7.88
2	21.15	53.07	95.19	0.041	76.41
3	20.65	31.78	95.65	0.15	70.70

[a] ***Caco2:*** *Permeability through cells derived from human colon adenocarcinoma; Caco2 values < 4 nm/sec (low permeability), values from 4 to 70 nm/ sec (medium permeability) and values > 70 nm/sec (high permeability). [24]*

[b] ***MDCK****: Permeability through Madin–Darby canine kidney cells; MDCK [25].*
values < 25 nm/sec (low permeability), values from 25 to 500 nm/sec (medium permeability) and values > 500 nm/sec (high permeability).

[c] ***HIA:*** *Percentage human intestinal absorption; HIA values from 0 to 20% (poorly absorbed), values from 20 to 70% (moderately absorbed) and values from 70 to 100% (well absorbed).*

[d] ***BBB:*** *Blood–brain barrier penetration; BBB values < 0.1 (low CNS penetration), values from 0.1 to 2 (medium CNS absorption) and values > 2 (high CNS absorption)*

[e] ***PPB:*** *Plasma protein binding; PPB values < 90% (poorly bound) and > 90% (strongly bound).*

CONCLUSION

Through this study it was concluded that expired drugs can be useful in the manufacture of chemical compounds that have an impact on pathogenic microorganisms when the active substance was separated from INH and interacted with chemical compounds such as ACAC, benzyl aldehyde and p-methoxy benzaldehyde by simple reaction Schiff base. So, these the target compounds were evaluated for antibacterial biological activity. We have noticed that these compounds are very effective against gram negative and gram-positive bacteria. So, recycling and separating expired drugs has a beneficial effect in discovering drugs.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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