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Prediction of Synergistic Antibiotic Combinations by Graph Learning

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Antibiotic resistance is a major public health concern. Antibiotic combinations, offering better efficacy at lower doses, are a useful way to handle this problem. However, it is difficult for us to find effective antibiotic combinations in the vast chemical space. Herein, we propose a graph learning framework to predict synergistic antibiotic combinations. In this model, a network proximity method combined with network propagation was used to quantify the relationships of drug pairs, and we found that synergistic antibiotic combinations tend to have smaller network proximity. Therefore, network proximity can be used for building an affinity matrix. Subsequently, the affinity matrix was fed into a graph regularization model to predict potential synergistic antibiotic combinations. Compared with existing methods, our model shows a better performance in the prediction of synergistic antibiotic combinations and interpretability.

Keywords: antibiotic combination, antimicrobial resistance, graph learning, bacterial infection, synergy effect

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INTRODUCTION

Antibiotic resistance is a growing health crisis, and it is emerging globally (Author Anonymous, 2013; Zhabiz et al., 2014; Murray et al., 2022). This crisis has been ascribed to the wide use and even abuse of antibiotics in the clinic, as well as a lack of economic incentives and market regulation of new antibiotic development (Ventola, 2015; Farha et al., 2021). An increasing number of Big Pharma have stopped developing new antibiotics, and the number of new FDA-approved antibiotics has gradually decreased since the 1980s (Ventola, 2015). Therefore, we have to find an alternative way to address this pressing public health problem.

Antibiotic combinations offer an effective strategy to combat antibiotic resistance (Tyers and Wright, 2019; Lv et al., 2021). Generally, antibiotic combinations can be divided into three groups: synergy, additive, and antagonism (Cokol et al., 2011). Synergistic antibiotic combinations are often used in clinics because they can offer better efficacy at lower doses (Mathers, 2015). In the microbiology laboratory, synergy or antagonism is usually identified through the fractional inhibitory concentration index (FICI) (Odds, 2003). However, this approach is expensive and time-consuming. To date, more than 300 antibiotics have been discovered (Wright, 2014), generating at least 44, 850 drug pairs. In addition, the efficacies of antibiotic combinations were also affected by doses (Maan et al., 2021), metabolic conditions (Cokol et al., 2018), and bacterial strains (Chandrasekaran et al., 2016). Consequently, millions of drug pairs need to be tested. As a result, it is impossible to screen synergistic antibiotic combinations by experimental approaches. Recently, with the development of artificial intelligence, many researchers have started to use computational approaches to identify synergistic drug combinations (Sheng et al., 2017; Weinstein et al., 2017). They used drug structures (Mason et al., 2017; Mason et al., 2018) and chemo-genomics

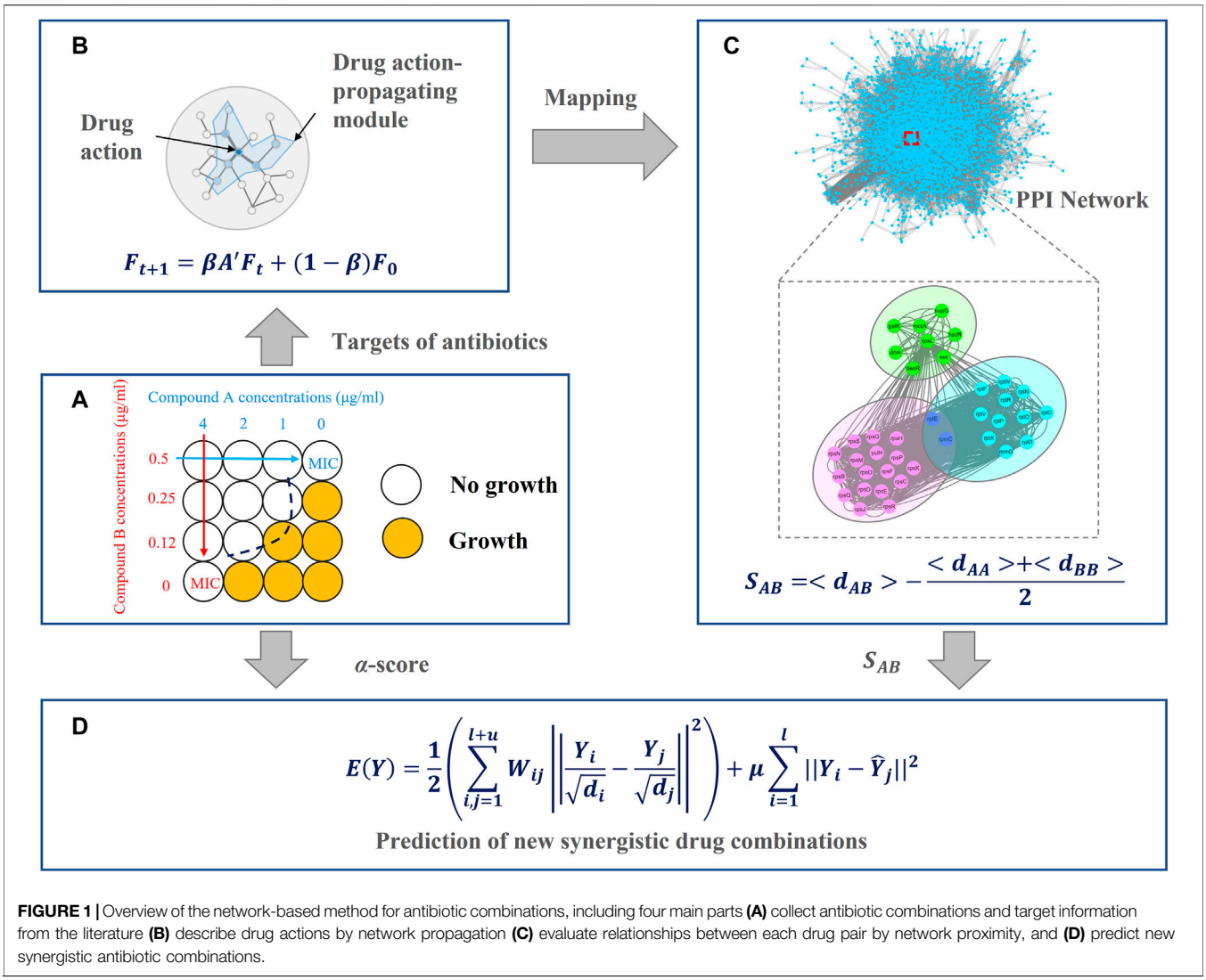

FIGURE 1 | Overview of the network-based method for antibiotic combinations, including four main parts **(A)** collect antibiotic combinations and target information from the literature **(B)** describe drug actions by network propagation **(C)** evaluate relationships between each drug pair by network proximity, and **(D)** predict new synergistic antibiotic combinations.

data (Chandrasekaran et al., 2016) as input to the "black-box" machine learning model to predict potential synergistic drug combinations. Although these models have shown good performance (Chandrasekaran et al., 2016; Mason et al., 2017; Mason et al., 2018), some limitations still exist. First and foremost, the curse of dimensionality is a serious problem. Specifically, the number of features (chemogenomic data: 3,979 and Morgan fingerprint: 2048) is much greater than the number of training sets (approximately 100). Furthermore, some features [e.g., chemo-genomics (Nichols et al., 2011)] are not only difficult to obtain but also hard to use to explain the mechanisms of the synergy effect. Therefore, more effective and interpretable features are needed.

Network pharmacology is a new paradigm for drug discovery (Hopkins, 2008) that can help us better understand intricate relationships between drugs, targets, pathways, and diseases (Menche et al., 2015; Cheng et al., 2019; Wang J. et al., 2021; Wang Y. et al., 2021; Li et al., 2021). In network pharmacology, the actions of drugs are regarded as perturbations to the network (Csermely et al., 2013). When a node is perturbed, neighboring nodes will also be affected (Saraswathi et al., 2009). However, perturbation experiments are expensive and time-consuming (Nichols et al., 2011). In this study, we introduced a network propagation method to simulate perturbation patterns of drug pairs (**Figure 1B**). Intuitively, variations in the medication regimen (synergy or antagonism) cause them to have a slight difference in the network structure and dynamics. Subsequently, we used the network proximity method (**Figure 1C**) to quantify the relationships between the interactomes between targets of different drugs. We found that synergistic antibiotic combinations tend to have smaller network proximity. In other words, network proximity is a good parameter to classify drug pairs and to avoid the curse of dimensionality. Finally, we introduced a mechanism-driven graph regularization model to predict synergistic antibiotic combinations based on this finding (**Figure 1D**). The results demonstrated that our method outperformed other existing methods in the prediction of synergistic antibiotic combinations and interpretability.

MATERIALS AND METHODS

In this section, we introduced the architecture of our model, as shown in **Figure 1**. In **Figure 1A**, we collected antibiotic combinations and their targets from the literature. In **Figure 1B**, the targets of these antibiotics were fed into the network propagation model. When a node is perturbed, neighboring nodes will also be affected, resulting in a subnetwork. We named this subnetwork as a drug action-propagating module (DAPM). In **Figure 1C**, we used a network proximity model to quantify the relationships between drug pairs. In **Figure 1D**, the network proximity of each drug pair was converted to an affinity matrix. This affinity matrix and the known antibiotic combinations were employed to build a graph regularization model, thereby predicting new synergistic antibiotic combinations.

Constructing the Protein–Protein Network and Drug–Target Network

We constructed the PPI network of *Escherichia coli* based on the STRING database version 11.5 (Szklarczyk et al., 2020). The interactions with a score less than 0.7 were ignored. The ultimate network included 59, 496 interactions involving 4, 020 proteins.

We collected drug–target interactions from previous literature reports or the DrugBank database (Wishart et al., 2017). Since we used the data from the *in vitro* antimicrobial test, proteins from bacteria were considered and proteins of *Homo sapiens* were ignored in this study.

Collecting Pairwise Antibiotic Combinations

In this study, we focused on pairwise antibiotic combinations by recent experimental data of the *Escherichia coli* strain MG1655 (Chandrasekaran et al., 2016). Interactions were quantified based on the α-score, and the following three types were used: synergy (α-score ≤ −0.25), additive (−0.25< α-score < 1), and antagonism (α-score ≥ 1) (Cokol et al., 2011). In this study, we only considered antibiotics with known targets (protein or RNA). In total, 91 pairwise antibiotic combinations involving 14 antibiotics were retained.

Network Propagation of Drug Action

Targets of the aforementioned antibiotics were fed into a network propagation model (Vanunu et al., 2010) to simulate the propagation of drug-action effects on the PPI network:

$$F_{t+1} = \beta A' F_t + (1-\beta)F_0, \tag{1}$$

where β is a parameter ($0 \le \beta \le 1$). Vanunu et al., (2010) confirmed that the algorithm is not sensitive to the choice of β as long as it is above 0.5, so we set β to 0.7. $A' = D^{-1/2}AD^{-1/2}$ in which D is a diagonal matrix, and the values of the diagonal element (d_{ii}) are equal to the degree of the vertexes (k_i), and A is an adjacency matrix. F_0 denotes a matrix, in which "1" indicates that the drug can bind to this target, and if the drug can bind multiple targets (n), the values were set as $1/n$. During each iteration, nodes can not only receive the information from their neighbors (first term) but can also retain their initial information (second term), resulting in a DAPM. Next, let us show that formula (1) converges. The general term formula of formula (1) is

$$F_t = \left(\beta A'\right)^t F_0 + (1-\beta)\sum_{i=0}^{t-1}\left(\beta A'\right)^i F_0 \tag{2}$$

Since $0 < \beta < 1$ and the eigenvalues of A' are in the range of −1 to 1 (according to the Perron–Frobenius theorem),

$$\lim_{t\to\infty}\left(\beta A'\right)^t = 0 \text{ and } \lim_{t\to\infty}\sum_{i=0}^{t-1}\left(\beta A'\right)^i = \left(I-\beta A'\right)^{-1} \tag{3}$$

Hence,

$$\lim_{t\to\infty} F_t = \left(I-\beta A'\right)^{-1} \tag{4}$$

Quantification of Relationships Between Each Drug Pair

Subsequently, the Jaccard index (**Eq. 5**) and network proximity model (**Eq. 6**) (Menche et al., 2015; Cheng et al., 2019) were used to quantify the relationships of each DAPM:

$$J_{AB} = \frac{|S_A \cap S_B|}{|S_A \cup S_B|} \tag{5}$$

where S_A and S_B are the nodes of drug A and drug B in their DAPMs, respectively.

$$S_{AB} \equiv < d_{AB} > - \frac{< d_{AA} > + < d_{BB} >}{2} \tag{6}$$

where $< d_{AA} >$ and $< d_{BB} >$ are the mean shortest distances between each pair of nodes in the DAPM. $< d_{AB} >$ is the mean shortest distance between each pair of nodes between the DAPM of drug A and the DAPM of drug B:

$$< d_{AB} > = \frac{1}{||A|| + ||B||}\sum_{y\in A} min_{x\in B} d(x,y) \tag{7}$$

where A and B are the DAPMs of drug A and drug B, respectively. $d(x, y)$ is the shortest distance between node x and node y. In the next section, we demonstrated how to build the affinity matrix and graph regularization model based on network proximity.

Prediction of Synergistic Antibiotic Combinations Based on Graph Regularization

Given three drugs (drug A, drug B, and drug C), if drug A–drug B is a synergistic antibiotic combination and drug A and drug C are pharmacologically similar, then drug C–drug B will likely be a synergistic antibiotic combination. Therefore, we can define a loss function:

TABLE 1 | List of antibiotics used for network analysis and their targets and mechanisms.

Drug	Abbreviation	Targets	Mechanism of action
Amikacin	AMK	rpsL Lin et al. (2018)	Protein synthesis, 30 S inhibition
Gentamicin	GEN	rpsL Lin et al. (2018)	Protein synthesis, 30 S inhibition
Tobramycin	TOB	rpsL Lin et al. (2018)	Protein synthesis, 30 S inhibition
Tetracycline	TET	rpsG, rpsN Buck and Cooperman (1990)	Protein synthesis, 30 S inhibition
Clarithromycin	CLA	rplD, rplV Salehi et al. (2020)	Protein synthesis, 50 S inhibition
Erythromycin	ERY	rplD, rplV Wekselman et al. (2017)	Protein synthesis, 50 S inhibition
Chloramphenicol	CHL	rplP Pongs et al. (1973)	Protein synthesis, 50 S inhibition
Ciprofloxacin	CIP	gyrA, parC Pan et al. (1996)	DNA gyrase inhibition
Levofloxacin	LEV	gyrA, parC Onodera et al. (2002)	DNA gyrase inhibition
Nalidixic acid	NAL	gyrA Shen and Pernet (1985)	DNA gyrase inhibition
Trimethoprim	TRI	folA Wróbel et al. (2020)	Folic acid biosynthesis inhibition
Oxacillin	OXA	dacB, ftsI Kocaoglu and Carlson (2015)	Cell wall
Cefoxitin	CEF	mrcA, mrcB, dacB, dacA, dacC, pbpG, ftsI Kocaoglu and Carlson (2015)	Cell wall
Nitrofurantoin	NIT	nfsA Aracena et al. (2014)	Multiple mechanisms

$$E(Y) = \frac{1}{2}\left(\sum_{i,j=1}^{l+u} W_{ij} \left\| \frac{Y_i}{\sqrt{d_i}} - \frac{Y_j}{\sqrt{d_j}} \right\|^2 \right) + \mu \sum_{i=1}^{l} \left\| Y_i - \hat{Y}_i \right\|^2 \quad (8)$$

where Y and $\hat{Y}$ are the entire and the known drug combination matrix, respectively. μ ($\mu > 0$) is the regularization parameter. d_i is the degree of node i. The key to this model rests on the construction of the affinity matrix W, which is calculated by **Eq. 9**.

$$W_{ij} = \begin{cases} 1, & -1 < S_{ij} < 0; \\ 0, & otherwise \end{cases} \quad (9)$$

where S_{ij} is the network proximity (**Eq. 6**) between drug i and drug j. The classifying model is as follows.

$$Y^* = argmin\, E(Y) \quad (10)$$

We then take the derivation of $E(Y)$ with respect to Y to solve the optimization problem.

$$\left.\frac{\partial E(Y)}{\partial Y}\right|_{Y=Y^*} = Y^* - QY^* + \mu\left(Y^* - \hat{Y}\right) = 0 \quad (11)$$

where $Q = D^{-1/2}WD^{-1/2}$. The detailed derivation of **Eq. 11** can be found in the supporting information, and the analytical solution of **Eq. 11** is

$$Y^* = \delta(I - \gamma Q)^{-1}\hat{Y} \quad (12)$$

where I is the identity matrix, $\gamma = {}^{1}/_{1+\mu}$, and $\delta = {}^{\mu}/_{1+\mu}$.

Performance Evaluation Metrics

The performance of the graph regularization model was estimated using the precision (**Eq. 13**), recall (**Eq. 14**), accuracy (**Eq. 15**), and F1 (**Eq. 16**), and these evaluation metrics can be defended as follows:

$$precision = \frac{TP}{TP + FP} \quad (13)$$

$$recall = \frac{TP}{TP + FN} \quad (14)$$

$$accuracy = \frac{TP + TN}{TP + FP + TN + FN} \quad (15)$$

$$F1 = \frac{2 \times precision \times recall}{precision + recall} \quad (16)$$

where TP, FP, FN, and TN are true positive, false positive, false negative, and true negative, respectively.

RESULTS

The Data Set of Antibiotic Combinations

We used previously reported antibiotic combinations involving 14 antibiotics (Chandrasekaran et al., 2016) listed in **Table 1**. These antibiotics range over various mechanisms of action, including protein biosynthesis, DNA and RNA replication, folate metabolism, and cell wall biosynthesis. Since we concentrated on the subtle differences among synergy, additive, and antagonism, all 91 pairwise combinations fall into three categories, according to the α-score (**Supplementary Table S1**). Targets of these antibiotics were collected from previous literature studies (Pongs et al., 1973; Shen and Pernet, 1985; Buck and Cooperman, 1990; Pan et al., 1996; Onodera et al., 2002; Aracena et al., 2014; Kocaoglu and Carlson, 2015; Wekselman et al., 2017; Lin et al., 2018; Salehi et al., 2020; Wróbel et al., 2020). Because some antibiotics are RNA-targeted small molecules, ribosomal proteins that affect antibiotic binding are considered targets of antibiotics. For example, 30S ribosomal proteins S7 (rpsG) and S14 (rpsN) were shown to be the most important for tetracycline binding (Buck and Cooperman, 1990). Mutations of 50S ribosomal proteins L22 (rplV) and L4 (rplD) will lead to macrolide (erythromycin, etc.) resistance (Wekselman et al., 2017).

Network analysis showed that the shortest distance between targets of antibiotic combinations ranged from 0 to 5 (**Supplementary Figure S1**). Most antibiotic combinations (92.3%) did not share the same targets. Approximately thirty percent of antibiotic combinations were adjacent, and almost half of synergistic antibiotic combinations (57.1%)

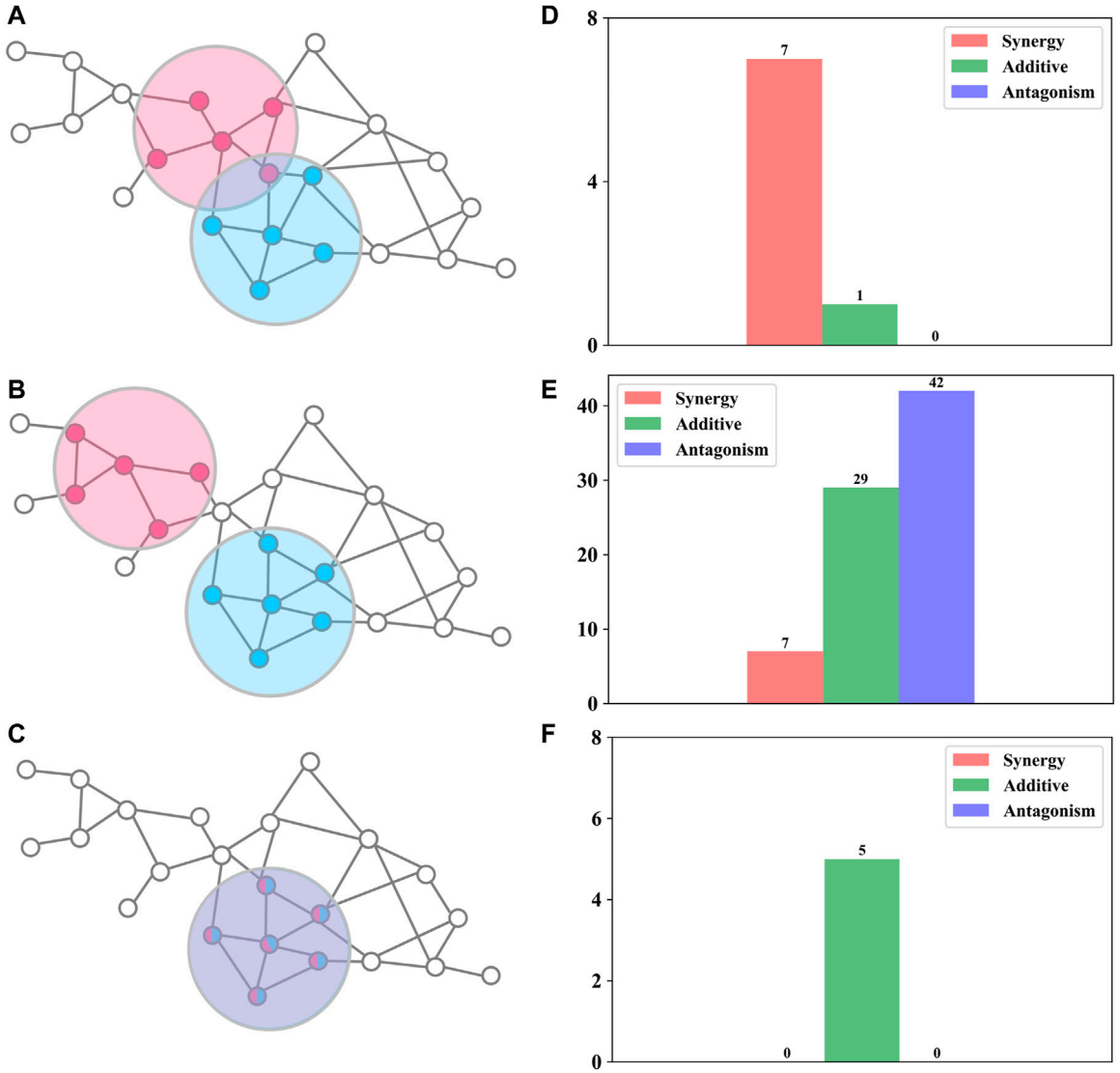

FIGURE 2 | Relationships between drug interactions and network structures **(A–C)** Sketch map of the three topologically distinct classes **(D–F)** The number of synergistic, additive, and antagonistic drug combinations for the corresponding network structure.

were included (**Supplementary Figure S1**). However, a considerable portion of antagonistic or additive antibiotic combinations have adjacent targets, but they are not synergistic (**Supplementary Figure S1**). Therefore, mere knowledge of the network structure may not be sufficient to explain the intricate interactions among antibiotic combinations and their targets. To investigate the network-based relationship between antibiotic combinations and their targets, we introduced network propagation (Vanunu et al., 2010) to predict the effect of antibiotics and antibiotic combinations on the PPI network.

Network Propagation of Drug Actions

Network propagation has been used to quantify the influence of mutations in colorectal tumorigenesis (Shin et al., 2017). When a mutation arises in a node, perturbation spreads out along the protein–protein interaction (PPI) network and eventually forms a mutation-propagating module. Similar to mutation, if a drug acts on a node, neighboring nodes are also affected (**Figure 1B**) (Saraswathi et al., 2009). Predictably, the impact is greatest in its neighbors, whereas nodes far away from targets receive attenuated influences. Therefore, we can generate a subnetwork with drug targets as hubs, and the nodes ($F_i^* \geq 0.0065$) will be incorporated into the subnetwork.

Based on the network propagation method (**Eq. 1**), these antibiotics and antibiotic combinations were mapped to the PPI network to investigate the potential relationships of these subnetworks (**Figure 1**). On average, DAPMs include approximately 13 nodes, although almost all drugs only have 1 to 2 targets. Because of the high threshold, each

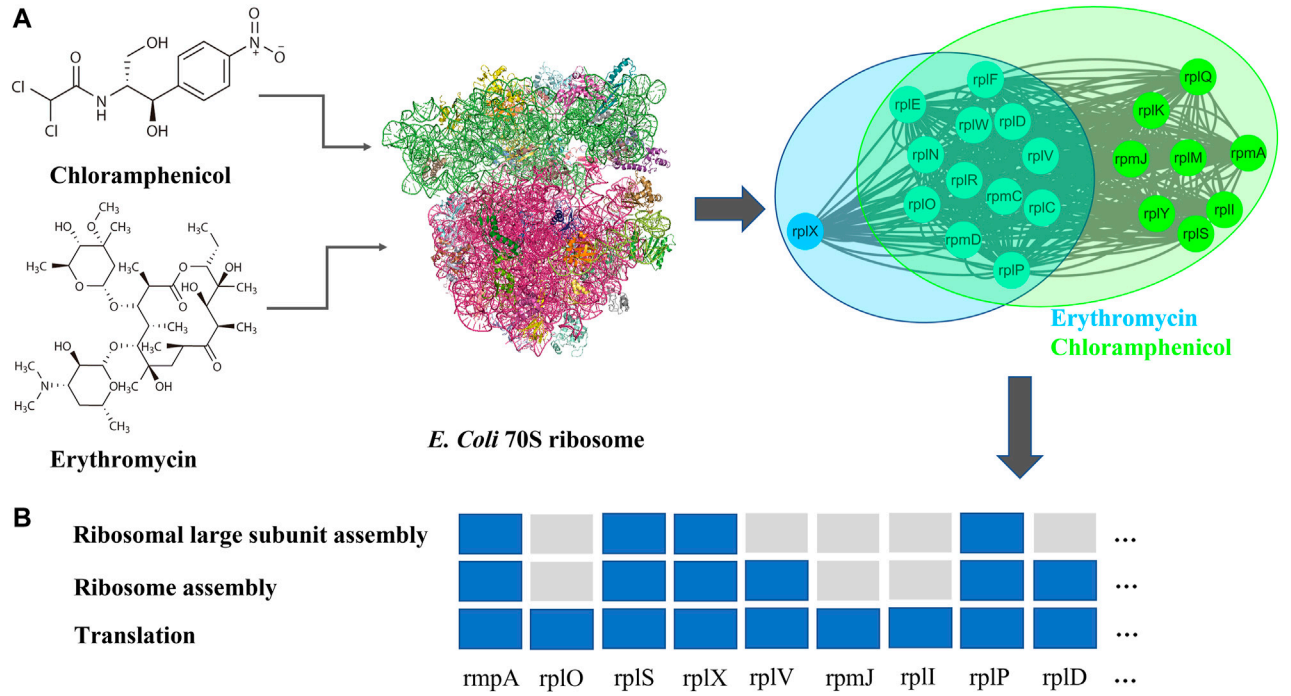

FIGURE 3 | (A) Chemistry structural formula, targets (PDB ID: 4V48), and DAPMs of chloramphenicol and erythromycin **(B)** gene enrichment analysis (Mi et al., 2018) for DAPMs of chloramphenicol and erythromycin.

DAPM consisted almost exclusively of nearest neighbors. Interestingly, we found that there are areas of overlap for some antibiotic combinations and that antibiotic combinations are associated with the synergy effect (**Figure 1C**). Hence, we inferred that the structure of DAPKs can be used to quantify interactions between drug pairs and thereby predict synergetic antibiotic combinations.

Network-Based Relationship Between DAMPs

Network proximity was used to investigate FDA-approved drug combinations (Cheng et al., 2019) and herb combinations in traditional Chinese medicine (Wang Y. et al., 2021; Zhang et al., 2021). Compared with random herb pairs, herb pairs in traditional Chinese medicine formulas tend to have smaller network proximity (Wang Y. et al., 2021). To probe whether it could also be used to distinguish synergy, additive, and antagonism, we used the Jaccard index (**Eq. 5**) and network proximity (**Eq. 6**) to quantify DAMP–DAMP interactions. We found that all possible antibiotic combinations can be divided into three topologically distinct categories: a) overlap: two DAMPs overlap but do not equate (**Figure 2A**); b) separation: two DAMPs are topologically separated (**Figure 2B**); and c) identical: two DAMPs are completely consistent (**Figure 2C**).

For overlap (**Figures 2A,D**), these antibiotic combinations are probably synergetic (87.5%, $p - value = 0.118$, permutation test). From the perspective of network pharmacology, if DAMPs of two drugs overlap, it indicates that the two drugs are pharmacologically similar (Cheng et al., 2019). For example, chloramphenicol and erythromycin both target the 50S ribosome, and their binding sites are the peptidyl transferase center (PTC) and the nascent peptide exit tunnel (NPET) on the 50S subunit, respectively (Lin et al., 2018). They can inhibit protein synthesis in a synergistic way (**Figure 3B**) (Chandrasekaran et al., 2016). As shown in **Figure 3A**, DAMPs of chloramphenicol and erythromycin have common nodes. Hence, the network proximity of the two DAMPs is negative, $S_{TET-CHL} = -0.97$. To verify this idea, we performed virtual screening for nodes in the DAMP of trimethoprim (a dihydrofolate reductase inhibitor). Eventually, we identified a dihydropteroate synthase inhibitor—sulfamethoxazole. The DAMPs between sulfamethoxazole and trimethoprim overlap ($S_{TRI-SUL} = -0.12$, **Supplementary Figure S2A**). Previous studies have shown that a combination of trimethoprim and sulfamethoxazole not only interferes with folic acid synthesis synergistically (Yeh et al., 2006) but also reduces the risk of bacterial resistance (Pappas et al., 2009). In summary, synergistic drug combinations tend to act on the same biological pathways.

For separation (**Figures 2B,E**), these antibiotic combinations were almost not synergetic (90.1%, $p - value < 10^{-4}$, permutation test, see more from SI). In other words, the two drugs are pharmacologically distinct in this case. For example, nalidixic acid (an inhibitor of DNA gyrase) and chloramphenicol (an inhibitor of protein synthesis) take effect in different biological processes, so

TABLE 2 | List of antibiotics used for the validation set and their targets and mechanisms.

Drug	Abbreviation	Targets	Mechanism of action
Kanamycin	KAN	rpsL Lin et al. (2018)	Protein synthesis, 30 S inhibition
Penicillin G	PNG	pbpG, dacB Kocaoglu and Carlson (2015)	Cell wall
Roxithromycin	ROX	rplD, rplV Salehi et al. (2020)	Protein synthesis, 50 S inhibition

their DAMPs are topologically separated ($S_{NAL-CHL} = 0.92$, **Supplementary Figure S2B**), and nalidixic acid and chloramphenicol do not show the synergy effect. Levofloxacin not only inhibits DNA gyrase but also inhibits DNA topoisomerase (**Table 1**). Hence, the DAMPs of levofloxacin and nalidixic acid overlap, resulting in the synergy effect. In addition, DAMPs of some synergistic drug combinations are topologically separated. This may result from the following reasons: a) experimental data itself: the correlation coefficient of the α-score between two replicates is only 0.81, which leads to a random error; b) some drugs have unknown targets: recent evidence suggests that gentamicin has a second binding site around H69 of the 23S rRNA of the 50S ribosome (Serio et al., 2018). This may be the reason for the synergy between gentamicin and tetracycline.

For identical (**Figures 2C,F**), these antibiotic combinations showed a definite additive effect (100%). For example, clarithromycin and erythromycin not only act on the same targets (**Table 1**) but also have similar chemical structures (98.1%, Tanimoto similarity; more details can be found in SI). Hence, we consider the two drugs to be pharmacologically identical, which leads to an additive effect.

To demonstrate the usefulness of the PPI network, an ablation test was performed where the PPI network was randomized. **Supplementary Figure S3** shows that the randomized PPI network produces worse results, so an accurate PPI network is crucial for our model.

Prediction of Synergistic Antibiotic Combinations by Graph Regularization

Graph regularization is a useful model to predict different relationships between various types of biological entities (Luo et al., 2018; Ding et al., 2020). Through the aforementioned analysis, we found that if two drugs are pharmacologically similar, then the drug pair is probably a synergistic antibiotic combination (**Figure 2D**). Therefore, we can define a loss function of Y (**Eq. 8**). However, if two drugs are pharmacologically identical ($S_{AB} = -1$), then the drug pair shows an additive effect (**Figure 2C**). Therefore, we set W_{ij} of these drug pairs to 0 (**Eq. 9**). Next, we used the aforementioned 14 antibiotics (**Table 1**) for the training set to predict interactions with the following three antibiotics (**Table 2**) by **Eq. 12**. The entire predicted scores are listed in **Table 3**. A larger predicted score of drug pairs suggests that they would probably be the synergistic antibiotic combinations. In **Supplementary Tables S2–S6**, we confirmed that the algorithm is not sensitive to the choice of γ, so it was simply fixed at 0.7. In **Supplementary Figure S4**, we demonstrated that the impact of the threshold changes on the performance of our method. When the threshold increases from 0.1 to 0.5, the precision increases, and the recall and

TABLE 3 | The entire predicted scores were calculated by a graph regularization model and synergistic antibiotic combinations are colored red.

Drug1	Drug2	Score	Drug1	Drug2	Score
KAN	AMK	0	PNG	CIP	0
KAN	GEN	0	PNG	LEV	0
KAN	TOB	0	PNG	NAL	0
KAN	TET	0	PNG	TRI	0.259
KAN	CLA	0	PNG	OXA	0.519 Mason et al. (2017)
KAN	ERY	0	PNG	CEF	0.519 Mason et al. (2017)
KAN	CHL	0	PNG	NIT	0
KAN	CIP	0	ROX	AMK	0.080
KAN	LEV	0	ROX	GEN	0.162
KAN	NAL	0	ROX	TOB	0
KAN	TRI	0	ROX	TET	0.485 Mason et al. (2017)
KAN	OXA	0 Mason et al. (2017)	ROX	CLA	0.405 Mason et al. (2017)
KAN	CEF	0	ROX	ERY	0.405 Yilancioglu (2019)
KAN	NIT	0	ROX	CHL	0.485 Yilancioglu (2019)
PNG	AMK	0	ROX	CIP	0
PNG	GEN	0	ROX	LEV	0
PNG	TOB	0	ROX	NAL	0
PNG	TET	0.259 Mason et al. (2017)	ROX	TRI	0
PNG	CLA	0	ROX	OXA	0.162
PNG	ERY	0 Mason et al. (2017)	ROX	CEF	0 Mason et al. (2017)
PNG	CHL	0	ROX	NIT	0

TABLE 4 | Performance comparison of CosynE (Mason et al., 2017), INDIGO (Chandrasekaran et al., 2016), and our model.

	Precision	Recall	Accuracy	F1
CosynE	0.83	0.38	0.86	0.53
INDIGO	0.3	0.85	0.58	0.44
Our model	0.875	0.7	0.90	0.78

accuracy decrease. When the threshold is larger than 0.2, the F1 decreases. Therefore, we set the threshold to be 0.2 in our model. Eight potential synergistic antibiotic combinations were found: TET-ROX, ROX-CLA, OXA-PNG, CEF-PNG, ROX-ERY, ROX-CHL, PNG-TET, and PNG-TRI. In the experiments conducted by Mason et al. (Mason et al., 2017), TET-ROX, ROX-CLA, OXA-PNG, CEF-PNG, and PNG-TET were identified as synergistic antibiotic combinations, and ROX-ERY, ROX-CHL, and PNG-TRI were additive. However, as alluded to above, there are random errors in experimental measurements, which might have some impact on the classification of antibiotic combinations. As expected, we found that ROX-ERY and ROX-CHL were identified as synergistic antibiotic combinations in the experiments by Yilancioglu (Yilancioglu, 2019). This means that our model has good stability for the prediction of synergistic antibiotic combinations.

Comparison With Other Methods

Previously, there have been studies to predict synergistic antibiotic combinations through computational methods. In this section, we compared the performance of our model (**Eqs 13–16**) with other methods, such as CosynE (Mason et al., 2017) and INDIGO (Chandrasekaran et al., 2016) on the benchmark dataset. As shown in **Table 4**, our model achieved better performance in terms of various metrics.

DISCUSSION

To reduce the cost and time of high-throughput drug combination experiments, we proposed a graph learning framework (**Figure 1**) to predict potential synergistic antibiotic combinations. First, we collected antibiotic combinations (**Supplementary Table S1**) and their corresponding targets (**Table 1**) from the literature. Network analysis revealed that the shortest distance between targets of antibiotic combinations was not sufficient to classify synergistic antibiotic combinations (**Supplementary Figure S1**). Therefore, we proposed a network proximity method combined with network propagation to quantify the relationships of antibiotic combinations (**Figures 1B,C**). An important finding is that synergistic antibiotic combinations have a specific network topological relationship, that is, the overlap pattern (**Figure 2**). This suggests that synergistic antibiotic combinations tend to act on the same biological pathways. Using the antibiotic combination erythromycin and chloramphenicol as a case study, we confirmed that the network proximity of their DAMPs is negative (**Supplementary Table S1**), and they can inhibit protein synthesis in a synergistic way (**Figure 3B**). In addition, the network proximity of each drug pair can be fed into the graph regularization model (**Eq. 8**) to predict new synergistic antibiotic combinations. Most of the predicted synergistic antibiotic combinations have been proven by a series of experiments (**Table 3**).

Previously, chemo-genomics data (Chandrasekaran et al., 2016) or structural compound fingerprints (Mason et al., 2017) have been used to build machine learning models and thereby predict antibiotic interactions between drug pairs. Based on the concepts proposed by these models, many potential synergistic antibiotic combinations were predicted and validated. However, the performance of these two methods is moderate (**Table 4**) because of the curse of dimensionality. Compared to these two approaches, our model is based on a feature at deeper molecular levels, the network proximity of DAMPs, which provides a more elegant and efficient way to describe the relationship of drug pairs. This not only makes our model have better predictability (**Table 4**) but also allows our model to achieve better interpretability. Even so, there are some limitations in our model. First, we focused on the paired antibiotic combinations. In the future, we will also investigate high-order drug combinations. Second, the PPI network is crucial for our model (**Supplementary Figure S3**). In the current model, an undirected network was used, and next, we will adopt a directed and signed network, which may be useful for improving the performance of our model.

CONCLUSION

Antibiotic combinations provide a useful way to combat antibiotic resistance. In this study, we proposed a graph learning framework to understand the mechanisms of drug pairs and to predict synergistic antibiotic combinations. By quantifying the relationship between drug pairs based on the network proximity of DAMPs, a graph regularization model can identify potential synergistic antibiotic combinations. This allows us to explore the need for antibiotic combinations more effectively.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

JL, YJ, and YS designed the experiments and wrote the manuscript. GL and WG supervised and provided instructive advice. GL obtained funding.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.849006/full#supplementary-material

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