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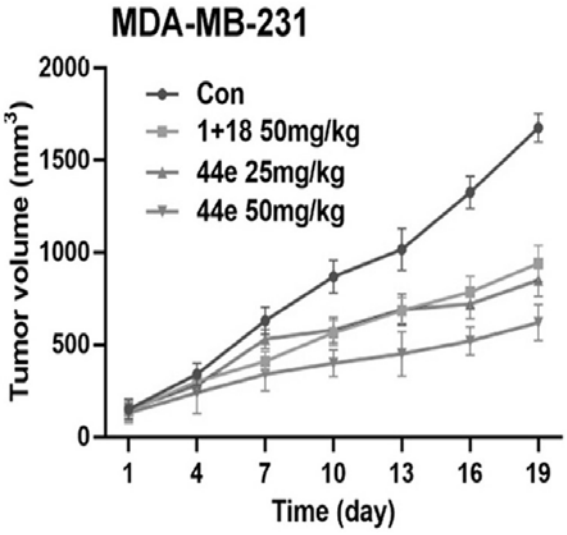
(54)发明名称

新型喹唑啉类小分子抑制剂及其在抗肿瘤药物中的应用

(57)摘要

本发明涉及一种双靶向BRD4-CK2小分子抑制剂及其在抗三阴性乳腺癌药物中的应用，属于抗肿瘤药学技术领域。本发明解决的技术问题是提供一种作为双靶向BRD4-CK2小分子抑制剂的化合物。该化合物包括如下所示的化合物及其药学上可接受的盐，本发明的化合物或其药学上可接受的盐，可以作为双靶向BRD4-CK2抑制剂，具有一定抗三阴性乳腺癌活性，能有效抑制三阴性乳腺癌细胞的生长。



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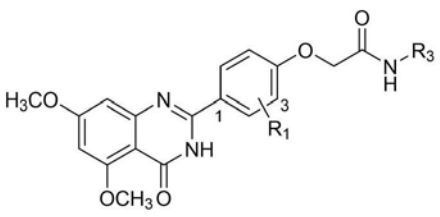
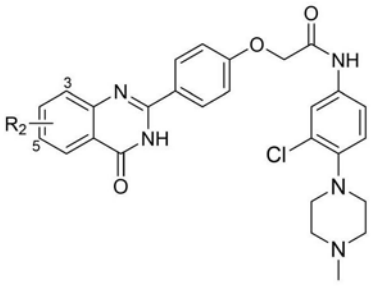
1.一种双靶向BRD4-CK2小分子抑制剂，其特征在于：结构式如式I或式II所示的化合物及其药学上可接受的盐：

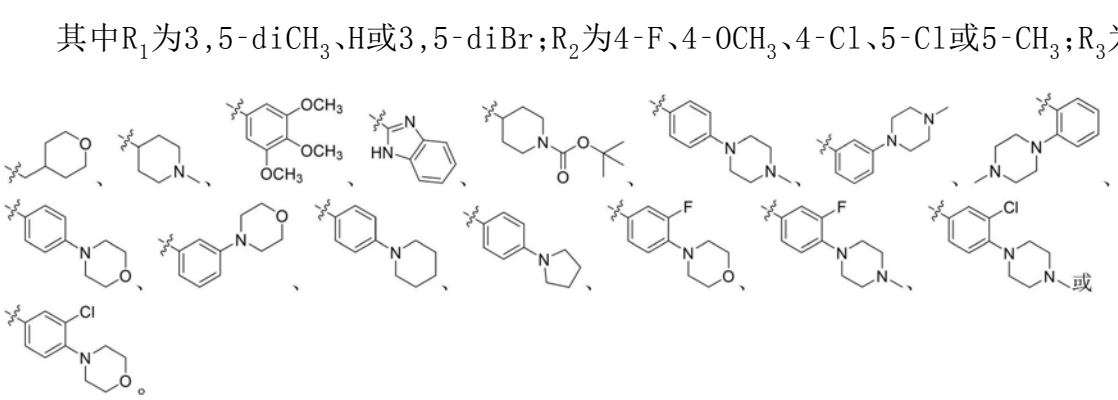


式I



式II

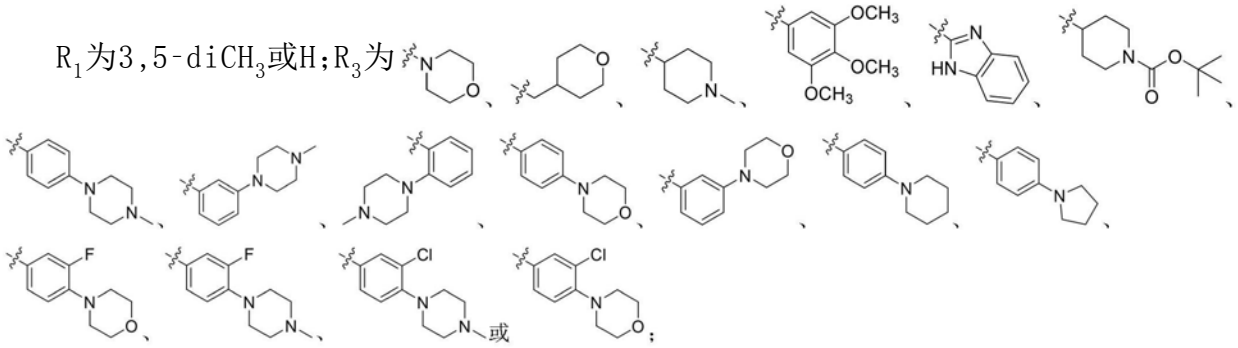
其中R_1为3,5-diCH_3、H或3,5-diBr；R_2为4-F、4-OCH_3、4-Cl、5-Cl或5-CH_3；R_3为



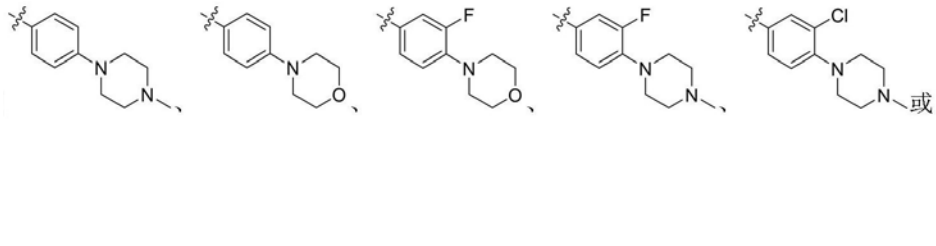
2.根据权利要求1所述的化合物或其药学上可接受的盐，其特征在于：结构式如式I所示。

3.根据权利要求2所述的化合物或其药学上可接受的盐，其特征在于：

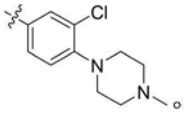
R_1为3,5-diCH_3或H；R_3为



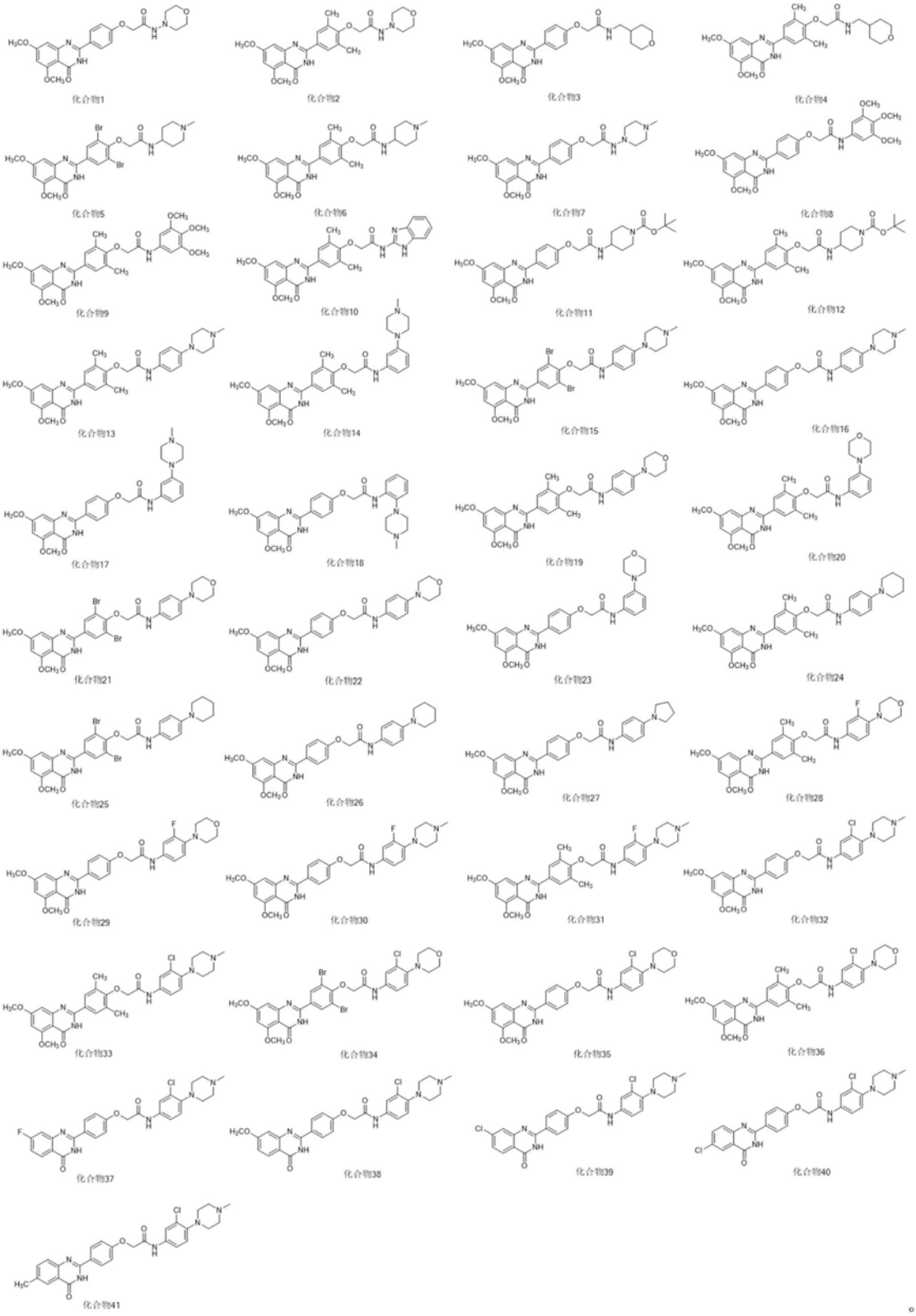
优选R_1为H；R_3为



更优选R_1为H；R_3为



4.根据权利要求1所述的化合物或其药学上可接受的盐，其特征在于：所述式I化合物为如下化合物：



5.权利要求1-4任一项所述的化合物或其药学上可接受的盐在制备三阴性乳腺癌治疗药物中的用途。

6.根据权利要求5所述的用途，其特征在于：所述三阴性乳腺癌治疗药物为抗肿瘤药物；优选所述抗肿瘤药物为双靶向BRD4-CK2抑制剂类药物。

7.一种药物组合物，其特征在于：它是包含有效剂量的权利要求1-6任一项所述的化合

物或其药学上可接受的盐的制剂。

新型喹唑啉类小分子抑制剂及其在抗肿瘤药物中的应用

技术领域

[0001] 本发明公开一种双靶向BRD4-CK2小分子抑制剂以及在肿瘤治疗中的应用，属于抗肿瘤药学技术领域。

背景技术

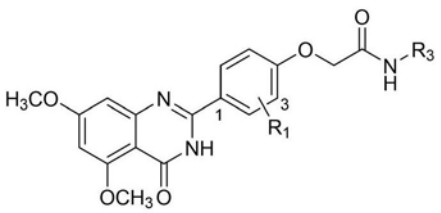
[0002] 据国际癌症研究中心最新统计表明，全球每年约有170万女性被确诊为乳腺癌，且导致50万患者死亡。在我国，近年来乳腺癌呈现大约4％的高速率增长，且有进一步年轻化的趋势，严重威胁女性的健康。三阴性乳腺癌(TNBC)是指雌激素受体(ER)，孕激素受体(PR)和人表皮生长因子受体2(Her-2)均为阴性的乳腺癌，占所有乳腺癌病理类型的15-20％，具有极高的致死率。TNBC由于其特殊的生物学行为和临床病理特征，其预后比其他类型的乳腺癌差，同时具有恶性程度高，发病年龄更加年轻，侵袭性高，容易复发转移等特点。由于ER，PR不表达，并缺乏HER2的扩增，TNBC通常没有公认的分子治疗靶标，临床上缺乏有效的治疗药物和治疗策略。

[0003] 目前临床上常用的TNBC治疗手段仍然是手术和常规的全身细胞毒化学治疗，治疗效果并不理想，预后依然很差。Bromodomain containing protein 4(BRD4)在癌症和其他慢性疾病中具有吸引力的表观遗传靶点。越来越多的BRD4抑制剂被发现并用于TNBC的治疗，但耐药性的出现是限制BRD4抑制剂临床应用最主要的难题。蛋白激酶casein kinase 2(CK2)是介导BRD4磷酸化和去磷酸化进而发挥作用的分子开关。此外，CK2抑制也会导致多药耐药细胞对已知药物摄取的增加。因此，设计合成双靶向BRD4-CK2的抑制剂或许可以为TNBC的治疗提供新的方案。

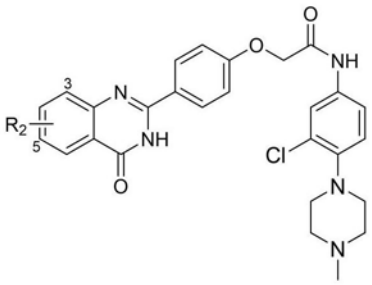
发明内容

[0004] 本发明解决的技术问题是提供一种作为双靶向BRD4-CK2抑制剂的新化合物。

[0005] 结构式如式I或式II所示的化合物及其药学上可接受的盐：

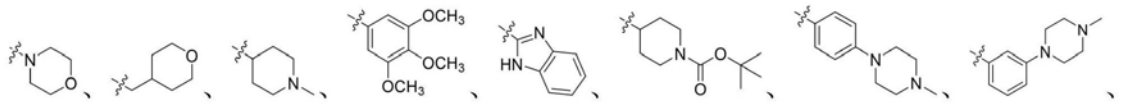


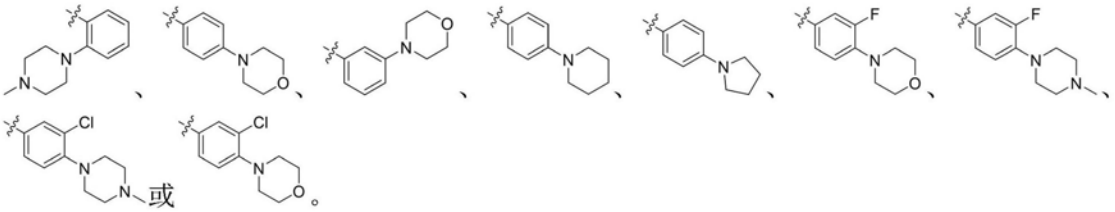
式I



式II。

[0006] 其中R_1为3,5-diCH_3、H或3,5-diBr；R_2为4-F、4-OCH_3、4-Cl、5-Cl或5-CH_3；R_3为





[0007] 本发明还提供上述化合物或其药学上可接受的盐在制备抗肿瘤药物中的用途。

[0008] 进一步的，所述抗肿瘤治疗药物优选为三阴性乳腺癌治疗药物。

[0009] 进一步的，所述三阴性乳腺癌治疗药物为双靶向BRD4-CK2小分子抑制剂，其用途为用于三阴性乳腺癌相关治疗。

[0010] 本发明还提供一种药物组合物，它是包含有效剂量的上述化合物或其药学上可接受的盐的制剂。

[0011] 本发明制备的化合物或其药学上可接受的盐，可以作为双靶向BRD4-CK2小分子抑制剂，具有较明显的三阴性乳腺癌治疗效果。

附图说明

[0012] 图1A为用自噬双标腺病毒GFP-mRFP-LC3转染MDA-MB-231细胞，5μM化合物32(44e)处理24小时，倒置显微镜下观查自噬通量变化图片。

[0013] 图1B 44e在0μM，2.5μM，5μM和10μM浓度下，MDA-MB-231细胞中抑制集落形成图片。

[0014] 图1C 44e在0μM，2.5μM，5μM和10μM浓度下，促进MDA-MB-231细胞死亡的图片。

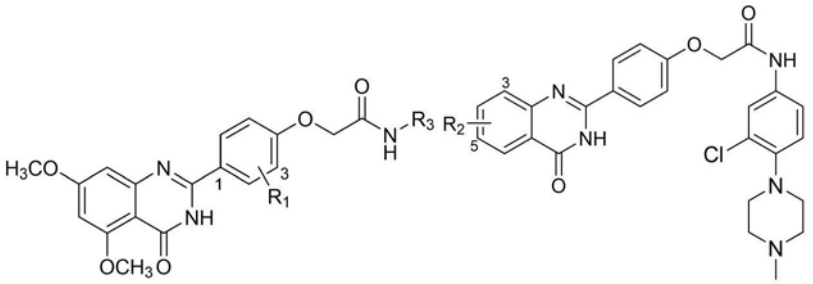
[0015] 图1D 44e在0μM和5μM浓度下，促进MDA-MB-231细胞凋亡的图片。

[0016] 图2A MDA-MB-231异种移植裸鼠模型通过口服给药44e，裸鼠肿瘤体积的变化图片。

[0017] 图2B MDA-MB-468异种移植裸鼠模型通过口服给药44e，裸鼠肿瘤体积的变化图片。

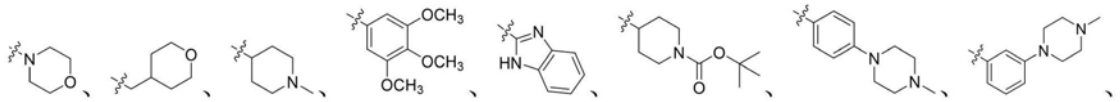
具体实施方式

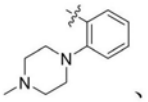
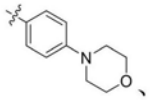
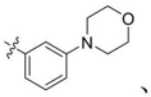
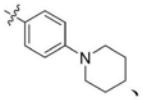
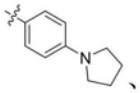
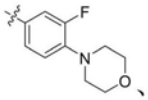
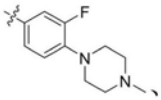
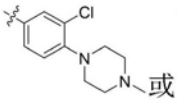
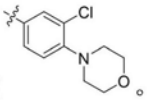
[0018] 本发明提供如式I或式II所示的化合物及其药学上可接受的盐：

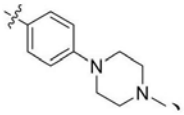
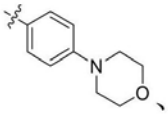
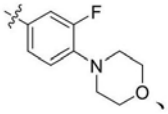
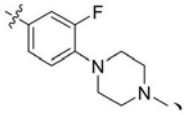
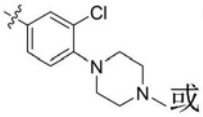
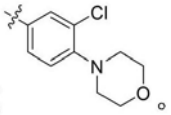


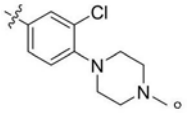
式I 式II。

[0019] 其中R_1为3,5-diCH_3、H或3,5-diBr；R_2为4-F、4-OCH_3、4-Cl、5-Cl或5-CH_3；R_3为

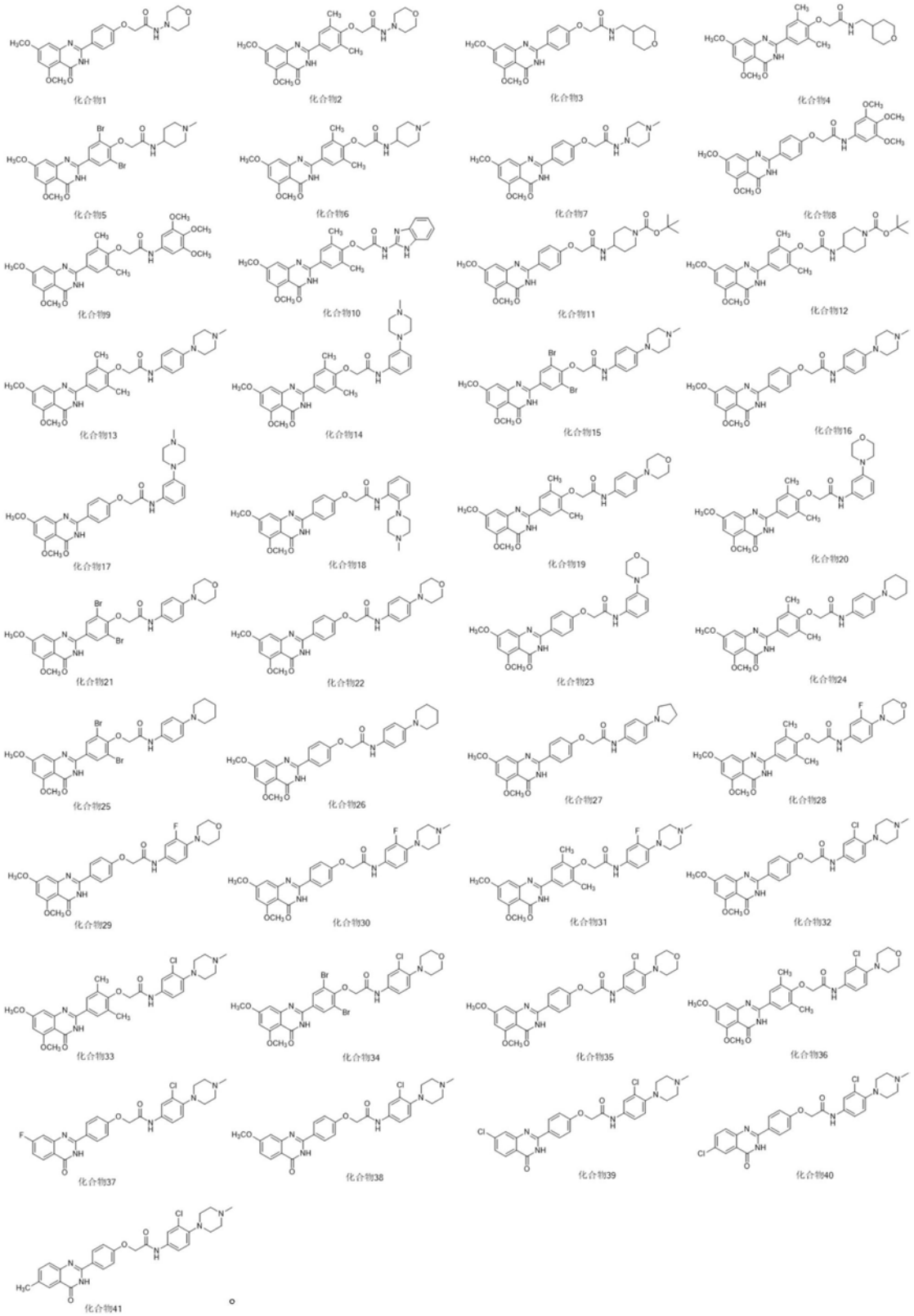


 、  、  、  、  、  、  、  或  。

[0020] 作为一个优选方案，优选R_1为H；R_3为  、  、  、  、  或  。

[0021] 更优选R_1为H;R_3为  。

[0022] 下面是本发明的化合物的一些优选结构。



[0023] 本发明还提供本发明所述的化合物的药学上可接受的盐。所述盐可以为硝酸盐、盐酸盐、硫酸盐或磷酸盐等。

[0024] 本发明还提供上述化合物或其药学上可接受的盐在制备治疗三阴性乳腺癌药物中的用途。进一步的，三阴性乳腺癌治疗药物优选为双靶向BRD4-CK2小分子抑制剂。

[0025] 本发明还提供一种药物组合物，它是包含有效剂量的上述化合物或其药学上可接

受的盐的制剂。可以通过本领域已知的方法可将本发明化合物制成以下形式：片剂、胶囊剂、水性或油性溶液剂、混悬剂、乳剂、乳膏剂、软膏剂，凝胶剂，喷鼻剂、栓剂、用于吸入的细小分散的粉剂或气雾剂或喷雾剂、用于胃肠道外(包括静脉内、肌内或输注)的无菌水性或油性溶液或混悬剂或无菌乳剂。可采用无菌水或水-丙二醇溶液作为溶剂来制备液体制剂，还可将活性组分配制在聚乙二醇水溶液中。用于口服给予的水性溶液可通过将活性组分溶解在水中并按需要加入合适的着色剂、矫味剂，稳定剂和增稠剂来制备。口服使用的水性混悬剂可通过将细小分散的活性组分与粘性物质一道分散在水中，所述粘性物质如为天然合成胶、树脂、甲基纤维素、羧甲基纤维素和其他药剂领域已知的悬浮剂。

[0026] 药物组合物可为单位剂量形式。在这些形式中，将所述组合物分成含适量活性组分的单位剂量。该单位剂量形式可为包装制剂，包装中包括分隔量的制剂，例如盒装片剂、胶囊剂和在管形瓶或安瓿中的粉剂。单位剂量形式还可为胶囊剂、扁囊剂或片剂或其可为适当数量的任何这些包装形式。

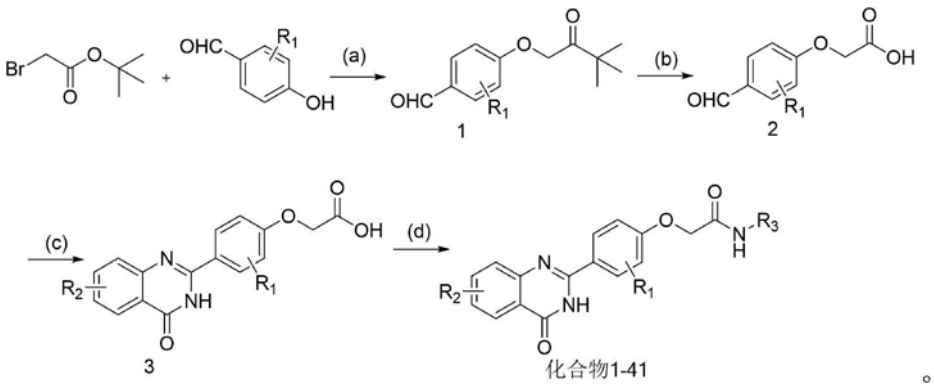
[0027] 本发明的药物组合物，其活性成分可仅为本发明的化合物，也可与其他抗三阴性乳腺癌化合物组合作为活性成分。

[0028] 在治疗三阴性乳腺癌时，可通过同时、序贯或单独给予各种治疗成分实现这种联合治疗。此类组合产品应用有效剂量范围内的本发明化合物和准许剂量范围内的其他药学活性剂。

[0029] 下面结合实施例对本发明的具体实施方式做进一步的描述，并不因此将本发明限制在所述的实施例范围之中。

[0030] 实施例1化合物1-41的合成。

[0031] 化合物1-41采用如下反应式合成：



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[0032] Reagents and conditions：(a)K_2CO_3，DMF，80℃，3h；(b)DCM，TFA，r.t，2h.；(c)DMAC，PTSA，$NaHSO_3$，120℃，4-8h；(d)DMF，Et_3N，HOBt，EDCI，r.t.；24h。

[0033] 中间体1a-c合成通法。

[0034] 溴乙酸叔丁酯(3.0mL，1.2equiv)和对羟基苯甲醛类似物(2.5g，1equiv)溶于DMF(10mL)，加入5.2g K_2CO_3，在80℃下反应1.5h。反应冷却后，加水80mL，过滤，干燥得到粗产物，用硅胶柱层析对固体进行纯化(CH_2Cl_2/CH_3OH＝80∶1-5∶1)，得到中间体1a-c(收率55-60％)，无需进一步纯化。

[0035] 中间体(1a)^{1}H-NMR(400MHz，CD_3OD)，δ(ppm)：9.84(1H，s)，7.86(2H，d，J＝8.8Hz)，7.06(2H，d，J＝8.8Hz)，4.71(2H，s)，1.48(9H，s)。HRMS(ESI)$^+$Calculated for $C_{13}H_{17}O_3$，[M+H]$^+$：m/z 221.1178，found 221.1170。

[0036] 中间体2a-c合成通法。

[0037] 中间体1a-c(2g,9mmol)溶于CH_2Cl_2(5mL),滴加TFA(1.5mL),常温反应1h。反应液减压浓缩得到白色固体中间体2a-c(72-77%收率),无需进一步纯化。

[0038] 中间体(2a)^{1}H-NMR(400MHz,CD_3OD),δ(ppm):12.28(1H,s),9.82(1H,s),7.84(2H,d,J=8.8Hz),7.04(2H,d,J=8.8Hz),3.68(2H,s)。HRMS(ESI)$^+$Calculated for $C_9H_9O_4$,[M+H]$^+$:m/z 181.0501,found 181.0507。

[0039] 中间体3a-h制备。

[0040] 将中间体2a(0.9mmol,1equiv)和苯甲醛衍生物(0.9mmol,1equiv)溶于DMAc(15mL)中,加入$NaHSO_4$(1.05mmol,1.2equiv)和PTSA(0.22mmol,0.24equiv)。将反应混合物在120℃下搅拌回流4-6h。反应完毕后,将水(100mL)加入反应,过滤得到沉淀。用硅胶柱层析对固体进行纯化(CH_2Cl_2/CH_3OH=80∶1-10∶1),分别得到中间体3a-h(收率41-65%)。

[0041] 中间体(3a)^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.97(1H,s),8.13(2H,d,J=8.8Hz),7.04(2H,d,J=8.8Hz),6.72(1H,d,J=2.0Hz),6.51(1H,d,J=2.0Hz),4.79(2H,s),3.88(3H,s),3.84(3H,s)。HRMS(ESI)$^+$Calculated for $C_{18}H_{16}N_aN_2O_6$,[M+N_a]$^+$:m/z 379.0906,found 379.0910。

[0042] 化合物1-41。

[0043] 中间体3a-h(0.42mmol,1.2eq)溶于DMF(3mL),0℃加入HOBT(0.54mmol,1.3equiv)搅拌10min,加入EDCI(0.54mmol,1.3equiv),然后加入含有氨基取代的反应物,继续反应,加入TEA(1.5mmol,3.5equiv)0℃反应1h,最后常温反应24h,反应液加水80mL,过滤干燥得到粗产物。用硅胶柱层析对固体进行纯化(CH_2Cl_2/CH_3OH=30∶1-1∶1),分别得到终产物1-41(收率43-66%)。

[0044] 化合物1,mp 262-264℃,yield 54.4%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):9.29(1H,s),8.90(1H,s),8.09-8.15(2H,m),7.08(1H,d,J=8.8Hz),7.02(1H,d,J=8.8Hz),6.71-6.73(1H,m),6.53(1H,d,J=2.4Hz),5.00(1H,s),4.56(2H,s),3.89(3H,s),3.85(3H,s),3.62(4H,t,J=4.4Hz),2.79(4H,t,J=4.4Hz);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):170.0,167.2,166.5,164.1,163.1,157.8,143.1,133.0,132.8,118.9,116.4,116.3,103.7,99.8,95.8,67.2,67.1,65.6,57.2,57.0,56.8,56.0。HRMS(ESI)$^+$Calculated for $C_{22}H_{24}N_aN_4O_6$,[M+N_a]$^+$:m/z 463.1594,found463.1601。

[0045] 化合物2,mp 265-267℃,yield 48.1%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):9.39(1H,s),8.81(1H,s),7.92(2H,s),6.69(1H,s),6.45(1H,s),4.60(1H,s),4.25(2H,s),3.87(3H,s),3.63(3H,s),3.64(4H,t,J=4.4Hz),2.82(4H,t,J=4.4Hz),2.30(6H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):169.3,164.8,163.6,160.7,158.1,157.4,153.7,130.4(2),128.3(2),128.2,105.0,100.7,97.0,70.5,65.9(2),55.8,55.5,54.5(2),16.1(2)。HRMS(ESI)$^+$Calculated for $C_{24}H_{29}N_4O_6$,[M+H]$^+$:m/z 469.2087,found469.2103。

[0046] 化合物3,mp 253-255℃,yield 60.3%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.93(1H,s),8.16(3H,d,J=8.8Hz),7.07(2H,d,J=8.8Hz),6.71(1H,d,J=2.4Hz),6.52(1H,d,J=2.4Hz),4.59(2H,s),3.89(3H,s),3.84(3H,s),3.79-3.81(2H,m),3.23(2H,t,J=8.8Hz),3.03(2H,t,J=8.8Hz),1.49-1.52(2H,m);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):167.9,166.8,164.0,162.8,157.8,157.6,142.9,132.6(2),118.4,116.1(2),

103.4,99.5,95.6,67.8,67.6(2),57.1,55.6,44.9,35.8,31.2(2)。HRMS(ESI)$^+$Calculated for $C_{24}H_{28}N_3O_6$,$[M+H]^+$:m/z454.1978,found454.1978。

[0047] 化合物4,mp 224-226℃,yield 47.2%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.78(1H,s),8.24(1H,t,J=6.0Hz),7.90(2H,s),6.73(1H,d,J=2.4Hz),6.51(1H,d,J=2.4Hz),4.28(2H,s),3.88(3H,s),3.84(3H,s),3.26(2H,t,J=11.6Hz),3.09(2H,t,J=7.6Hz),2.31(6H,s),1.55(2H,d,J=8.8Hz),1.16-1.19(2H,m);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):168.1,166.5,162.5,160.8,157.4,157.3,143.0,132.5(2),131.0(2),125.0,103.4,99.4,95.7,71.3,67.4(2),57.0,56.5,44.6,35.5,31.1(2),16.5(2)。HRMS(ESI)$^+$Calculated for $C_{26}H_{31}N_aN_3O_6$,$[M+N_a]^+$:m/z 504.2111,found 504.2106。

[0048] 化合物5,mp 267-269℃,yield 45.5%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):9.50(1H,s),8.50(2H,s),6.80(1H,s),6.57(1H,s),4.48(2H,s),3.90(3H,s),3.86(3H,s),3.45-3.47(2H,m),3.07-3.08(2H,m),2.75-2.77(2H,m),2.97-2.99(2H,m);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.0,164.4,160.9,159.5,154.1,152.4,149.7,132.0(2),131.2,117.6(2),104.8,101.5,98.2,71.0,56.0,55.7,52.7(2),43.5,42.6,28.8(2)。HRMS(ESI)$^+$Calculated for $C_{24}H_{27}Br_2N_2O_5$,$[M+H]^+$:m/z 611.0328,found 611.0320。

[0049] 化合物6,mp 270-272℃,yield 43.0%。^{1}H-NMR(400MHz,$CDCl_3$),δ(ppm):7.78(1H,t,J=6.0Hz),6.78(1H,s),6.41(1H,s),4.27(2H,s),3.91(6H,s),2.80(2H,d,J=4.8Hz),2.29(6H,s),2.14(2H,t,J=8.8Hz),2.01-2.03(2H,m),1.57-1.60(2H,m);^{13}C-NMR(100MHz,$CDCl_3$),δ(ppm):167.0(2),165.0,164.8,161.0,157.2,154.1,131.1,128.5(2),125.1,123.0,105.0,101.2,98.2,70.6,56.2,55.7,54.5(2),46.3,45.8,32.3(2),16.5(2)。HRMS(ESI)$^+$Calculated for $C_{26}H_{33}N_4O_5$,$[M+H]^+$:m/z 481.2451,found481.2443。

[0050] 化合物7,mp 256-258℃,yield 62.5%。^{1}H-NMR(400MHz,$CDCl_3$+CD_3OD),δ(ppm):8.25(2H,d,J=2.4Hz),7.35(2H,d,J=2.4Hz),7.01(1H,s),6.72(1H,s),4.82(2H,s),4.16(3H,s),4.15(3H,s),3.09-3.12(4H,m),2.85-2.87(4H,m),2.57(3H,s);^{13}C-NMR(100MHz,$CDCl_3$+CD_3OD),δ(ppm):166.0,165.9,165.2,161.1,159.9,153.5,152.7,129.2(2),126.0,114.8(2),104.5,100.8,97.9,66.7,55.8,55.5,53.9(2),49.2(2),45.2。HRMS(ESI)$^+$Calculated for $C_{23}H_{27}N_aN_5O_5$,$[M+N_a]^+$:m/z 476.1910,found476.1904。

[0051] 化合物8,mp 269-271℃,yield 63.7%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):10.07(1H,s),8.15(2H,d,d,J=8.8Hz),7.13(2H,d,J=8.8Hz),7.06(2H,s),6.74(1H,d,J=2.4Hz),6.53(1H,d,J=2.4Hz),4.80(2H,s),3.89(3H,s),3.85(3H,s),3.74(6H,s),3.62(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.0,164.4,161.0,160.7,159.5,153.0,152.7(2),151.7,134.5,133.7,129.7(2),124.2,114.7(2),104.3,100.2,97.6,97.4(2),67.1,60.1(2),56.0,55.7(2)。HRMS(ESI)$^+$Calculated for $C_{27}H_{27}N_aN_3O_8$,$[M+N_a]^+$:m/z 544.1696,found 544.1694。

[0052] 化合物9,mp 240-242℃,yield 54.9%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):9.98(1H,s),7.92(2H,s),7.18(2H,s),6.76(1H,d,J=2.4Hz),6.54(1H,d,J=2.4Hz),4.48(2H,s),3.89(3H,s),3.85(3H,s),3.75(6H,s),3.63(3H,s),2.36(6H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.3,164.4,161.0,159.5,157.8,152.8,152.7(2),152.0,134.5,133.8,130.9(2),128.6(2),127.3,104.5,100.6,97.7,97.6(2),71.0,60.1,56.0,55.8(2),55.7,

16.2(2)。HRMS(ESI)$^+$Calculated for $C_{29}H_{31}N_aN_3O_8$,$[M+N_a]^+$:m/z 572.2009,found 572.2007。

[0053] 化合物10,mp 257-260℃,yield 57.2%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):7.93(2H,s),7.45-7.47(2H,m),7.10-7.11(1H,m),6.74(1H,d,J=2.4Hz),6.50(1H,d,J=2.4Hz),4.67(2H,s),3.89(3H,s),3.84(3H,s),2.37(6H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):168.3,164.2,160.9,160.1,159.7,159.1,158.0,153.1,152.7,146.6,130.6(2),130.4,128.7,128.4(2),128.0,121.2(2),104.7,101.1,97.5,70.9,55.9,55.6,16.3(2)。HRMS(ESI)$^+$Calculated for $C_{27}H_{26}N_5O_5$,$[M+Na]^+$:m/z 522.1753,found 522.1752。

[0054] 化合物11,mp 244-246℃,yield 64.4%。^{1}H-NMR(400MHz,$CDCl_3$),δ(ppm):8.26(2H,d,J=8.4Hz),7.06(2H,d,J=8.4Hz),6.46(1H,d,J=2.4Hz),6.42(1H,d,J=8.4Hz),4.56(2H,s),3.98(3H,s),3.93(3H,s),4.03-4.06(4H,m),2.87(2H,t,J=12.4Hz),1.94(2H,t,J=12.4Hz),1.45(9H,s);^{13}C-NMR(100MHz,$CDCl_3$),δ(ppm):166.9,165.4,161.9,161.5,159.9,154.7,152.2,129.8(2),115.0(2),104.9,101.1,98.5,79.9,67.5,56.6,55.9,46.7,42.8,42.5,32.1(2),28.6(5)。HRMS(ESI)$^+$Calculated for $C_{28}H_{35}N_4O_7$,$[M+H]^+$:m/z 539.2506,found539.2514。

[0055] 化合物12,mp 236-239℃,yield 63.8%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):8.13(1H,d,J=8.4Hz),7.90(2H,s),6.72(1H,d,J=2.4Hz),6.48(1H,d,J=2.4Hz),4.26(2H,s),3.91-3.93(2H,m),3.89(3H,s),3.83(3H,s),3.31-3.33(2H,m),2.80-2.82(2H,m),2.29(6H,s),1.70-1.72(2H,m),1.39(9H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.8,164.0,161.9,160.9,160.5,157.6,153.9,153.2,152.9,130.6(2),128.3(2),104.8,100.9,97.4,78.6,70.8,55.9,55.5,45.7,42.7,42.2,31.2(2),28.1(3),16.1(2)。HRMS(ESI)$^+$Calculated for $C_{30}H_{39}N_4O_4$,$[M+Na]^+$:m/z 589.2638,found 589.2635。

[0056] 化合物13,mp 240-243℃,yield 60.3%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.86(1H,s),9.86(1H,s),7.92(2H,s),7.55(2H,d,J=8.8Hz),6.90(2H,d,J=8.8Hz),6.74(1H,d,J=2.0Hz),6.51(1H,d,J=2.0Hz),4.47(2H,s),3.88(3H,s),3.84(3H,s),3.08(4H,t,J=4.8Hz),2.44(4H,t,J=4.8Hz),2.35(6H,s),2.21(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):165.9,164.3,161.0,159.8,157.8,153.1,152.4,147.6,130.8(2),130.2,128.4(2),127.9,121.1(2),115.6(2),104.7,101.2,97.6,71.2,56.0,55.7,54.6(2),48.5(2),45.8,16.8(2)。HRMS(ESI)$^+$Calculated for $C_{31}H_{36}N_5O_5$,$[M+H]^+$:m/z 558.2716,found 558.2719.

[0057] 化合物14,mp 237-240℃,yield 57.2%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.88(1H,s),10.01(1H,s),7.93(2H,s),7.42(1H,s),7.28(1H,t,J=8.0Hz),7.23(1H,t,J=8.0Hz),6.77(1H,d,J=2.4Hz),6.73(1H,s),6.51(1H,d,J=2.4Hz),4.49(2H,s),3.88(3H,s),3.84(3H,s),3.35-3.37(4H,m),3.17(4H,s),2.86(3H,s),2.36(6H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.4,165.6,164.2,160.9,159.7,157.6,153.0,152.4,149.9,139.2,130.7(2),129.3,128.3(2),127.9,111.7,107.5,104.6,101.1,97.6,71.0,57.8,55.9,55.6,52.2(2),45.7(2),16.2(2)。HRMS(ESI)$^+$Calculated for $C_{31}H_{35}NaN_5O_5$,$[M+Na]^+$:m/z 580.2536,found580.2534。

[0058] 化合物15,mp 270-272℃,yield 55.7%。^{1}H-NMR(400MHz,AceticAcid-d_4),δ

(ppm):8.58(2H,s),7.58(2H,d,J=8.8Hz),7.22(2H,d,J=8.8Hz),7.05(1H,d,J=2.0Hz),6.71(1H,d,J=2.0Hz),5.01(2H,s),4.10(3H,s),4.11(3H,s),3.92(4H,t,J=4.8Hz),3.41(4H,t,J=4.8Hz),3.12(3H,s);^{13}C-NMR(100MHz,AceticAcid-d_4),δ(ppm):168.7,167.8,164.9.0,163.1,156.1,154.8,151.0,148.8,133.9(2),132.9,132.4,123.9(2),119.7(2),119.2(2),105.7,103.0,100.2,72.6,57.2,57.1,54.7(2),48.7(2),44.3。HRMS(ESI)$^+$ Calculated for $C_{29}H_{30}Br_2N_5O_5$,$[M+H]^+$:m/z 688.0593,found 688.0596。

[0059] 化合物16,mp 267-269℃,yield 58.2%。^{1}H-NMR(400MHz,AceticAcid-d_4),δ(ppm):8.31(2H,d,J=8.0Hz),7.82(2H,d,J=8.0Hz),7.39(2H,d,J=8.0Hz),7.23(2H,d,J=8.0Hz),7.12(1H,s),6.72(1H,s),5.00(2H,s),4.12(3H,s),4.09(3H,s),3.94(4H,brs),2.44(4H,brs),3.16(3H,s);^{13}C-NMR(100MHz,AceticAcid-d_4),δ(ppm):169.6,167.9,165.0,163.2,162.2,154.9,154.4,148.8,132.5,131.6(2),126.8,124.2(2),119.2(2),116.8(2),105.4,102.3,100.0,68.8,57.2,57.1,54.6(2),48.7(2),44.4。HRMS(ESI)$^+$ Calculated for $C_{29}H_{32}N_5O_5$,$[M+H]^+$:m/z530.2403,found 530.2401。

[0060] 化合物17,mp 256-258℃,yield 66.0%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.95(1H,s),10.12(1H,s),8.17(2H,d,J=8.4Hz),7.38(1H,s),7.21(1H,t,J=8.0Hz),7.11-7.15(2H,m),6.77(1H,dd,J=8.0,2.4Hz),6.72(1H,d,J=2.4Hz),6.52(1H,d,J=2.0Hz),4.82(2H,s),3.85(3H,s),3.82(3H,s),3.74-3.76(2H,m),3.51-3.53(2H,m),3.16(2H,s),2.96-2.98(2H,m),2.86(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.8,166.6,164.7,161.5,160.9,153.5,152.9,150.5,139.8,129.9(2),125.5,115.1(2),112.3,112.0,107.8,105.0,101.5,97.9,89.7,67.5,56.0,55.7,52.7(2),46.2(2),42.5。HRMS(ESI)$^+$ Calculated for $C_{29}H_{31}NaN_5O_5$,$[M+Na]^+$:m/z 552.2223,found 552.2222。

[0061] 化合物18,mp 252-255℃,yield 58.6%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):9.96(1H,s),8.23(2H,d,J=2.4Hz),8.15(1H,d,J=8.4Hz),7.14-7.28(4H,m),6.71(1H,d,J=2.0Hz),6.52(1H,d,J=2.0Hz),4.90(2H,s),3.89(3H,s),3.85(3H,s),3.51-3.53(4H,m),2.98-3.03(4H,m),2.87(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.0,164.3,161.1,160.0,159.9,158.7,152.8,152.5,141.0,132.0,129.8(2),125.5,125.3,124.8,120.8,114.8(2),104.6,100.9,97.5,67.4,56.0,55.6,53.3(2),48.5(2),42.4。HRMS(ESI)$^+$ Calculated for $C_{29}H_{31}NaN_5O_5$,$[M+Na]^+$:m/z 552.2223,found 552.2215。

[0062] 化合物19,mp 273-275℃,yield 61.8%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.87(1H,s),9.89(1H,s),7.92(2H,s),7.57(2H,d,J=8.4Hz),6.92(2H,d,J=8.4Hz),6.74(1H,d,J=2.0Hz),6.52(1H,d,J=2.0Hz),4.45(2H,s),3.89(3H,s),3.84(3H,s),3.73(4H,t,J=4.8Hz),3.05(4H,t,J=4.8Hz);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):166.4,165.3,161.6,159.5,157.8,155.4,145.9,142.0,131.5(2),129.9(2),123.4(2),121.1,121.0(2),119.5,103.5,98.5,97.1,71.2,65.0(2),56.4,56.1,52.0(2),16.3(2)。HRMS(ESI)$^+$Calculated for $C_{30}H_{33}N_4O_4$,$[M+H]^+$:m/z 545.2400,found 545.2400。

[0063] 化合物20,mp 264-267℃,yield 48.3%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.87(1H,s),9.91(1H,s),7.92(2H,s),7.34(1H,s),7.15-7.23(2H,m),6.74(1H,d,J=2.4Hz),6.69(1H,dd,J=8.4,2.4Hz),6.51(1H,d,J=2.8Hz),4.47(2H,s),3.88(3H,s),3.84(3H,s),3.73(4H,t,J=4.8Hz),3.08(4H,t,J=4.8Hz),2.35(6H,s);^{13}C-NMR(100MHz,DMSO-d_6),

δ(ppm):167.7,166.4,164.3,162.5,161.0,157.7,152.4,151.5,139.1,130.8(2),129.1,128.4(2),127.9,110.9(2),106.,104.6,101.2,97.6,71.0,66.1(2),56.0,55.7,48.5(2),16.2(2)。HRMS(ESI)$^+$Calculated for $C_{30}H_{32}N_aN_4O_6$,$[M+N_a]^+$:m/z 567.2220,found 567.2211。

[0064] 化合物21,mp 242-244℃,yield 49.7%。^{1}H-NMR(400MHz,DMSO-d_6+CF_3COOD),δ(ppm):10.34(1H,s),8.39(2H,s),7.87(2H,d,J=8.4Hz),7.62(2H,d,J=8.4Hz),6.80(1H,d,J=2.0Hz),6.62(1H,d,J=2.0Hz),4.74(2H,s),3.95(4H,t,J=4.8Hz),3.86(6H,s),3.58(4H,t,J=4.8Hz);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):166.6,166.4,162.6,156.9,156.8,153.8,139.9,138.6,134.6(2),122.5(2),122.2(2),118.6(2),116.8,115.0,104.5,99.8,98.4,72.4,65.0(2),57.0,56.6,55.4(2)。HRMS(ESI)$^+$Calculated for $C_{28}H_{27}Br_2N_4O_6$,$[M+H]^+$:m/z 675.0277,found 675.0275。

[0065] 化合物22,mp 276-278℃,yield 60.7%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.92(1H,s),9.93(1H,s),7.50(2H,d,J=8.8Hz),7.12(2H,d,J=8.8Hz),6.91(2H,d,J=8.8Hz),6.71(1H,d,J=2.4Hz),6.50(1H,d,J=2.4Hz),4.76(2H,s),3.88(3H,s),3.84(3H,s),3.72(4H,t,J=4.8Hz),3.05(4H,t,J=4.8Hz);^{13}C-NMR(100MHz,$CDCl_3$+CF_3COOD),δ(ppm):169.0,163.2,162.0,160.0,156.4,137.5,131.3,131.0,123.4,123.3,121.8,121.5,119.0,117.0,116.9,116.1,116.0,113.3,101.0,98.5,94.8,67.0,64.5,64.2,56.9,56.3,56.1(2)。HRMS(ESI)$^+$Calculated for $C_{28}H_{29}N_4O_4$,$[M+H]^+$:m/z 517.2087,found 517.2086。

[0066] 化合物23,mp 272-274℃,yield 51.0%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):10.02(1H,s),8.16(2H,d,J=8.4Hz),7.28(1H,t,J=2.4Hz),7.09-7.16(4H,m),6.72(1H,d,J=2.4Hz),6.68(1H,dd,J=8.4,2.4Hz),6.51(1H,d,J=2.4Hz),4.79(2H,s),3.88(3H,s),3.84(3H,s),3.72(4H,t,J=4.8Hz),3.06(4H,t,J=4.8Hz);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.1(2),164.3,161.0,160.5,152.9,152.5,151.5,139.2,129.4(2),129.2,125.0,114.6(2),110.9,110.7,106.4,104.5,100.9,97.5,67.1,66.1(2),56.0,55.6,48.5(2),。HRMS(ESI)$^+$Calculated for $C_{28}H_{29}N_4O_6$,$[M+H]^+$:m/z 517.2087,found 517.2053。

[0067] 化合物24,mp 265-267℃,yield 56.1%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.87(1H,s),9.58(1H,s),7.92(2H,s),7.53(2H,d,J=8.8Hz),6.89(2H,d,J=8.8Hz),6.74(1H,s),6.52(1H,s),4.45(2H,s),3.89(3H,s),3.84(3H,s),3.06-3.08(4H,m),2.35(6H,s),1.58-1.63(4H,m),1.51-1.52(2H,m);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):165.8,164.2,160.9,157.8,153.1,152.4,148.4,130.8(2),129.9,128.7(2),127.9,121.1(2),116.1(2),104.7,101.2,97.6,71.1,56.0,55.6,50.0(2),35.8,25.3(2),23.9,16.2(2)。HRMS(ESI)$^+$Calculated for $C_{31}H_{35}N_4O_5$,$[M+H]^+$:m/z 543.2607,found 535.2607。

[0068] 化合物25,mp 271-273℃,yield 62.0%。^{1}H-NMR(400MHz,DMSO-d_6+CF_3COOD),δ(ppm):10.48(1H,s),8.52(2H,s),7.94(2H,d,J=8.8Hz),7.73(2H,d,J=8.8Hz),6.85(1H,d,J=2.4Hz),6.65(1H,d,J=2.4Hz),4.78(2H,s),3.93(3H,s),3.91(3H,s),3.58-3.61(4H,m),1.94-1.97(4H,m),1.70(2H,s);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):166.0,165.4,165.3,161.7,155.2,151.8,142.1,139.8,138.2,133.2(2),122.4(2),121.5,121.4(2),118.0(2),104.6,99.8,98.9,71.7,56.8(2),56.5,56.1,23.8(2),21.0。

HRMS(ESI)$^+$Calculated for $C_{29}H_{29}Br_2N_4O_5$,[M+H]$^+$:m/z 673.0484,found 673.0490。

[0069] 化合物26,mp 253-256℃,yield 63.4%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.93(1H,s),9.89(1H,s),8.17(2H,d,J=8.8Hz),7.45(1H,d,J=8.8Hz),7.12(2H,d,J=8.8Hz),6.88(1H,d,J=8.8Hz),6.71(1H,d,J=2.4Hz),6.51(1H,d,J=2.4Hz),4.75(2H,s),3.88(3H,s),3.84(3H,s),3.04-3.07(4H,m),1.57-1.62(4H,m),1.50-1.51(2H,m);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):166.6,165.8,162.8,161.9,157.6,156.4,144.2,139.7,137.9,131.7(2),122.3(2),120.9,120.8(2),119.2,115.3(2),103.1,98.7,96.1,67.2,56.5(2),56.2,23.6(2),20.8。HRMS(ESI)$^+$Calculated for $C_{29}H_{31}N_4O_5$,[M+H]$^+$:m/z 515.2294,found 515.2300。

[0070] 化合物27,mp 243-245℃,yield 50.7%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):9.80(1H,s),8.16(2H,d,J=9.2Hz),7.42(2H,d,J=9.2Hz),7.14(2H,d,J=8.8Hz),6.72(1H,d,J=2.4Hz),6.50-6.53(2H,m),4.74(2H,s),3.89(3H,s),3.85(3H,s),3.17-3.19(4H,m),1.92-1.95(4H,m);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):166.2,163.2,162.1(2),157.3,156.9,143.3,141.4,132.0(2),121.1(2),119.9,119.8(2),118.6,115.5(2),103.0,98.9,95.6,67.4,56.8,56.3,55.3(2),23.9(2)。HRMS(ESI)$^+$Calculated for $C_{28}H_{29}N_4O_5$,[M+H]$^+$:m/z 501.2138,found 501.2133。

[0071] 化合物28,mp 258-260℃,yield 55.9%。^{1}H-NMR(400MHz,DMSO-d_6+CF_3COOD),δ(ppm):7.80(1H,dd,J=14.0,2.4Hz),7.68(2H,s),7.47(1H,dd,J=9.2,2.4Hz),7.36(1H,t,J=9.2Hz),6.83(1H,d,J=2.0Hz),6.55(1H,d,J=2.0Hz),4.46(2H,s),3.82-3.85(4H,m),3.81(3H,s),3.78(3H,s),3.34-3.36(4H,m),2.30(6H,s);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):169.2,168.8,164.3,162.6,159.3,158.9,155.8(250.2Hz),143.9,134.7(2),130.6(2),132.6(2),126.4,126.2,124.6,122.9,104.8,101.2,97.0,72.7,65.9,65.5,58.0,57.7,56.2(2),17.7(2)。HRMS(ESI)$^+$Calculated for $C_{30}H_{32}FN_4O_6$,[M+Na]$^+$:m/z 585.2125,found 585.2122。

[0072] 化合物29,mp 236-238℃,yield 58.0%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):10.1(1H,s),8.15(2H,d,J=8.8Hz),7.56(1H,dd,J=14.8,2.4Hz),7.32(1H,dd,J=14.8,2.4Hz),7.14(2H,d,J=14.8Hz),7.02(1H,t,J=9.6Hz),6.73(1H,d,J=2.4Hz),6.53(1H,d,J=2.4Hz),4.80(2H,s),3.89(3H,s),3.85(3H,s),3.71-3.74(4H,m),2.94-2.96(4H,m);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.1,164.5,161.1,160.7,159.6,155.6,153.2,153.0,151.8,135.7(40.6Hz),133.5(36.0Hz),129.7(2),124.3,119.2,115.8,114.7,108.2(100.8Hz),104.4,100.3,97.6,67.1,66.2(2),56.1,55.7,50.8(2)。HRMS(ESI)$^+$Calculated for $C_{28}H_{28}FN_4O_6$,[M+H]$^+$:m/z 535.1993,found 535.1992。

[0073] 化合物30,mp 245-247℃,yield 59.9%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):11.94(1H,s),10.16(1H,s),8.17(2H,d,J=8.8Hz),7.55(1H,dd,J=14.8,2.4Hz),7.30(2H,dd,J=8.8,2.4Hz),7.13(1H,d,J=2.4Hz),7.00(1H,t,J=8.8Hz),6.71(1H,d,J=2.4Hz),6.51(1H,d,J=2.4Hz),4.79(2H,s),3.88(3H,s),3.84(3H,s),2.96(4H,t,J=4.8Hz),2.46(4H,brs),2.22(3H,s);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):166.2,166.1,163.3,162.8,162.2,157.4,157.0,156.2,153.7,143.2,134.7(42.8Hz),134.5(36.8Hz),120.2,118.6,116.0,115.5(2),108.5(100.8Hz),103.1,98.9,95.5,67.4,56.8,56.7,56.4,56.3,

53.1(2),47.9。HRMS(ESI)$^+$Calculated for $C_{29}H_{31}FN_5O_5$,[M+H]$^+$:m/z 548.2309,found 548.2311。

[0074] 化合物31,mp 253-256℃,yield 61.4%。^{1}H-NMR(400MHz,$CDCl_3$),δ(ppm):8.60(1H,s),7.85(2H,s),7.58(1H,dd,J=14.0,2.4Hz),7.26-7.27(1H,m),6.94(1H,d,J=8.8Hz),6.81(1H,s),6.44(1H,s),4.40(2H,s),3.92(6H,s),3.10(4H,t,J=4.4Hz),2.61(4H,t,J=4.4Hz),2.36(9H,s);^{13}C-NMR(100MHz,$CDCl_3$),δ(ppm):166.1,165.2,161.4,157.0,156.7,154.2,153.9,152.3,137.3(36.4Hz),131.9(36.8Hz),131.3(2),129.1,128.5(2),119.1,115.9,109.2(56.4Hz),105.0,101.4,98.4,70.5,56.3,55.8,55.2(2),50.7(2),46.2,16.5(2)。HRMS(ESI)$^+$Calculated for $C_{31}H_{35}FN_5O_5$,[M+H]$^+$:m/z 576.2622,found 576.2624。

[0075] 化合物32,mp 273-276℃,yield 62.9%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):10.29(1H,s),8.17(2H,d,J=8.4Hz),7.85(1H,s),7.55(1H,d,J=8.8Hz),7.21(1H,d,J=8.8Hz),7.12(2H,d,J=8.8Hz),6.71(1H,s),6.50(1H,s),4.81(2H,s),3.88(3H,s),3.84(3H,s),3.30-3.37(8H,m),2.86(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.3,164.2,160.9,160.3,159.8,153.1,152.3,143.1,135.1,129.3(2),127.5,125.1,121.3(2),119.3,114.6(2),104.5,101.1,97.4,67.0,55.9,55.6,52.9(2),48.1(2),42.2。HRMS(ESI)$^+$Calculated for $C_{29}H_{31}ClN_5O_5$,[M+H]$^+$:m/z 564.2014,found 564.2011。

[0076] 化合物33,mp 260-263℃,yield 45.8%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):10.22(1H,s),7.93(2H,s),7.91(1H,d,J=2.4Hz),7.61(1H,dd,J=8.8,2.4Hz),7.14(1H,d,J=8.8Hz),6.73(1H,d,J=2.4Hz),6.50(1H,d,J=2.4Hz),4.49(2H,s),3.88(3H,s),3.84(3H,s),3.17(4H,brs),2.93(4H,brs),2.35(6H,s),2.23(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.6,164.1,160.9,157.6,153.3,153.0,145.0,134.3,130.7(2),128.4(2),127.4,121.7,120.8,119.6,104.8,101.0,97.4,79.2,71.0,56.0,55.6,54.9(2),51.1,48.6,45.8,16.3(2)。HRMS(ESI)$^+$Calculated for $C_{31}H_{35}ClN_5O_5$,[M+H]$^+$:m/z 614.2148,found 614.2174。

[0077] 化合物34,mp 268-271℃,yield 47.6%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):12.17(1H,s),10.21(1H,s),8.52(2H,s),7.88(1H,d,J=2.4Hz),7.56(1H,dd,J=8.8,2.4Hz),7.16(1H,d,J=8.8Hz),6.81(1H,s),6.56(1H,s),4.67(2H,s),3.89(3H,s),3.85(3H,s),3.74(4H,t,J=4.4Hz),2.94(4H,t,J=4.0Hz);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):165.1,164.4,160.9,154.0,152.4,149.8,144.8,134.3,132.0(2),131.3,127.4,122.8,121.7,120.9,119.7,117.7(2),104.8,101.5,98.2,71.3,66.4(2),56.1,55.7,51.5(2)。HRMS(ESI)$^+$Calculated for $C_{28}H_{25}Br_2ClNaN_4O_6$,[M+Na]$^+$:m/z 730.9707,found 730.9714。

[0078] 化合物35,mp 247-249℃,yield 56.0%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):10.20(1H,s),8.15(2H,d,J=8.8Hz),7.52(1H,dd,J=8.8,2.4Hz),7.13-7.16(3H,m),6.73(1H,d,J=2.4Hz),6.54(1H,d,J=2.4Hz),4.81(2H,s),3.89(3H,s),3.85(3H,s),3.73(4H,t,J=4.4Hz),2.92(4H,t,J=4.0Hz);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.1,164.4,161.1,160.6,159.5,153.0,151.8,144.6,134.4,129.7(2),127.5,124.3,121.4,120.9,119.3,114.7(2),104.3,100.3,97.6,67.0,66.4(2),56.0,55.7,51.5(2)。HRMS(ESI)$^+$Calculated for $C_{28}H_{27}ClNaN_4O_6$,[M+Na]$^+$:m/z 573.1517,found 573.1533。

[0079] 化合物36,mp 258-261℃,yield 51.5%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):10.17(1H,s),7.90(3H,s),7.62(2H,dd,J=8.8,2.4Hz),7.15(1H,d,J=8.8Hz),6.75(1H,d,J=2.4Hz),6.54(1H,d,J=2.4Hz),4.49(2H,s),3.88(3H,s),3.85(3H,s),3.73(4H,t,J=4.4Hz),2.93(4H,t,J=4.0Hz),2.35(6H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):165.5,164.4,161.0,159.5,157.9,152.9,151.9,144.7,134.4,130.9(2),128.6(2),127.4,127.2,121.7,120.8,119.6,104.5,100.5,97.7,71.0,66.4(2),56.0,55.7,51.5(2),16.2(2)。HRMS(ESI)$^{+}$Calculated for $C_{30}H_{32}ClN_4O_6$,$[M+H]^{+}$:m/z 579.2010,found 579.2018。

[0080] 化合物37,mp 255-257℃,yield 58.2%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):10.28(1H,s),8.18(2H,d,J=8.8Hz),7.87(1H,d,J=2.4Hz),7.76-7.80(2H,m),7.70(1H,td,J=8.8,2.4Hz),7.56(1H,dd,J=8.8,2.4Hz),7.22(1H,d,J=8.8Hz),7.15(1H,d,J=8.8Hz),3.52(2H,d,J=11.2Hz),3.38(2H,d,J=11.2Hz),3.19-3.22(2H,m),3.29(2H,t,J=13.2Hz),2.89(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.3,161.8,161.0,158.6,151.4,145.7,143.1,135.1,130.0(32Hz),129.4(2),127.5,125.3,123.1(97.2Hz),121.9(33.2Hz),121.3(2),119.3,114.7(2),110.6(93.2Hz),67.0,52.8(2),48.1(2),42.5。HRMS(ESI)$^{+}$Calculated for $C_{27}H_{25}ClFNaN_5O_3$,$[M+Na]^{+}$:m/z 544.1528,found 544.1526。

[0081] 化合物38,mp 243-245℃,yield 47.7%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):10.28(1H,s),8.03(2H,d,J=8.8Hz),7.85(1H,d,J=2.4Hz),7.80(1H,d,J=8.8Hz),7.60(1H,d,J=2.4Hz),7.51-7.56(2H,m),7.28(2H,d,J=8.8Hz),7.17(1H,d,J=8.8Hz),4.88(2H,s),3.90(3H,s),3.52(2H,d,J=12.0Hz),3.36(2H,d,J=12.0Hz),3.21(2H,td,J=12.0,2.8Hz),2.97(2H,td,J=12.0,2.8Hz),2.87(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.4,162.1,160.0(2),157.5,149.7,143.1,135.1,129.1(2),127.5,125.7,124.2,124.1,121.5.121.4,121.3,119.3,114.7(2),105.9,67.0,55.7,52.9(2),48.1(2),42.2。HRMS(ESI)$^{+}$Calculated for $C_{28}H_{29}ClN_5O_4$,$[M+H]^{+}$:m/z 534.1908,found 534.1899。

[0082] 化合物39,mp 281-283℃,yield 54.5%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):12.60(1H,s),10.30(1H,s),10.05(1H,s),8.20(2H,d,J=8.8Hz),8.06(1H,d,J=2.4Hz),7.86(1H,d,J=2.4Hz),7.83(1H,dd,J=8.8,2.4Hz),7.72(1H,d,J=8.8Hz),7.55(1H,dd,J=8.8,2.4Hz),7.22(1H,d,J=8.8Hz),7.15(2H,d,J=8.8Hz),4.83(2H,s),3.52-3.55(2H,m),3.38(2H,d,J=12.0Hz),3.18(2H,td,J=12.0,2.8Hz),2.99(2H,td,J=12.0,2.8Hz),2.89(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.3,161.4,160.5,152.3,147.6,143.1,135.1,134.6,130.3,129.6(2),129.5,127.5,125.2,124.9,121.9,121.3(2),119.3,114.7(2),67.0,52.9(2),48.1(2),42.2。HRMS(ESI)$^{+}$Calculated for $C_{27}H_{26}Cl_2N_5O_3$,$[M+H]^{+}$:m/z 538.1413,found 538.1408。

[0083] 化合物40,mp 280-282℃,yield 46.2%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):12.51(1H,s),10.18(1H,s),8.19(2H,d,J=8.8Hz),8.12(1H,d,J=8.8Hz),7.80(1H,d,J=2.4Hz),7.45(1H,d,J=2.4Hz),7.51(2H,dd,J=8.8,2.4Hz),7.13-7.17(3H,m),4.81(2H,s),3.33(4H,brs),2.91-2.93(4H,m),2.25(3H,s);^{13}C-NMR(100MHz,DMSO-d_6+CF_3COOD),δ(ppm):166.3,161.6,160.9,153.6,149.4,143.2,139.4,135.2,129.9(2),128.1,127.7,126.7,125.9,124.7,121.5,121.4,119.6,119.5,114.9(2),67.1,53.0(2),48.2(2),42.2。HRMS(ESI)$^{+}$Calculated for $C_{27}H_{26}Cl_2N_5O_3$,$[M+H]^{+}$:m/z 538.1413,found 538.1415。

[0084] 化合物41,mp 279-282℃,yield 55.5%。^{1}H-NMR(400MHz,DMSO-d_6),δ(ppm):12.33(1H,s),10.27(1H,s),9.91(1H,s),8.17(1H,d,J=8.8Hz),8.01(1H,d,J=8.4Hz),7.93(2H,s),7.85(1H,d,J=2.4Hz),7.56(1H,dd,J=8.8,2.4Hz),7.52(1H,s),7.31(1H,dd,J=8.4,2.4Hz),7.22(1H,d,J=8.8Hz),7.14(1H,d,J=8.8Hz),4.82(2H,s),3.52(2H,d,J=11.6Hz),3.38(2H,d,J=11.6Hz),3.20(2H,t,J=12.0Hz),2.97(2H,t,J=12.0Hz),2.88(3H,s),2.46(3H,s);^{13}C-NMR(100MHz,DMSO-d_6),δ(ppm):166.3,162.2,160.3,151.9,148.8,145.0,143.1,135.1,129.4(2),127.7,127.5,126.8,125.7,125.5,121.3(2),119.3,118.3,114.7(2),67.0,52.9(2),48.1(2),42.7,21.4。HRMS(ESI)$^{+}$Calculated for $C_{28}H_{29}ClN_5O_3$,$[M+H]^+$:m/z 518.1959,found 518.1953。

[0085] 试验例1化合物1-41在1μM浓度下对BRD4和CK2的抑制活性及对MDA-MB-231和MDA-MB-468细胞的抗增殖活性。

化合物	Kinase inhibitory activity (1 μM, %)[a]		Anti-proliferative active (IC_{50}, μM)[b]	
	BRD4	CK2	MDA-MB-231	MDA-MB-468
1	45.29 ± 1.42	42.20 ± 1.25	14.71 ± 1.55	12.54 ± 0.87
2	42.58 ± 0.82	40.31 ± 1.76	20.57 ± 0.62	18.50 ± 1.23
3	43.19 ± 3.47	43.43 ± 2.59	21.21 ± 2.80	26.61 ± 0.85
4	37.47 ± 1.42	33.70 ± 3.64	23.61 ± 0.58	25.56 ± 1.57
5	51.11 ± 3.47	47.43 ± 2.19	16.93 ± 0.87	14.81 ± 1.66
6	52.38 ± 1.15	49.23 ± 2.59	17.21 ± 2.80	15.55 ± 2.21
7	55.71 ± 2.02	53.64 ± 1.79	12.82 ± 1.72	13.65 ± 0.23
8	54.19 ± 1.28	47.43 ± 1.09	23.37 ± 1.35	15.03 ± 0.26
9	53.50 ± 3.47	42.14 ± 0.33	24.41 ± 2.81	16.55 ± 1.80
10	56.14 ± 3.09	25.61 ± 6.57	>50	30.31 ± 1.56
11	62.10 ± 0.52	55.68 ± 2.07	13.12 ± 1.67	16.62 ± 2.21
12	57.39 ± 2.06	51.38 ± 1.69	22.37 ± 2.30	17.24 ± 1.63
13	78.36 ± 4.07	69.31 ± 2.19	10.81 ± 1.85	12.59 ± 1.24
14	72.44 ± 2.87	57.08 ± 1.27	14.57 ± 1.64	15.74 ± 2.66
15	62.58 ± 0.52	58.47 ± 1.23	13.64 ± 2.17	15.83 ± 1.11
16	82.30 ± 2.73	80.02 ± 4.22	6.46 ± 0.62	8.80 ± 1.15
17	74.33 ± 1.28	56.02 ± 1.90	13.22 ± 0.54	8.07 ± 1.68
18	69.01 ± 2.30	51.44 ± 0.65	17.32 ± 0.79	11.50 ± 0.54
19	81.44 ± 1.15	63.61 ± 0.34	8.23 ± 1.39	9.49 ± 1.18
20	73.71 ± 0.83	55.73 ± 1.86	11.26 ± 0.93	13.44 ± 0.77
21	67.20 ± 1.52	58.29 ± 3.62	13.21 ± 2.21	15.11 ± 1.75
22	80.65 ± 3.98	75.39 ± 2.52	7.67 ± 1.03	9.54 ± 0.74
23	61.74 ± 2.03	60.02 ± 1.38	15.53 ± 0.91	17.80 ± 1.22
24	69.24 ± 3.67	70.51 ± 0.54	13.43 ± 2.53	9.43 ± 1.09
25	56.39 ± 0.46	62.47 ± 1.23	16.35 ± 2.02	15.63 ± 1.26
26	71.06 ± 2.96	75.20 ± 1.38	10.27 ± 2.03	11.63 ± 0.46
27	68.51 ± 1.72	67.52 ± 0.71	19.56 ± 1.63	7.82 ± 4.02
28	83.29 ± 3.45	76.21 ± 2.53	10.30 ± 1.23	9.16 ± 0.93
29	85.43 ± 2.65	79.61 ± 3.65	7.09 ± 0.25	8.58 ± 1.51
30	85.37 ± 1.58	82.46 ± 0.13	6.30 ± 2.37	7.55 ± 1.73
31	87.61 ± 2.73	80.23 ± 0.42	5.72 ± 1.45	8.33 ± 1.17
32	92.28 ± 0.66	90.65 ± 1.39	2.66 ± 0.81	3.52 ± 0.58
33	89.27 ± 1.47	85.40 ± 0.38	3.49 ± 1.23	4.58 ± 0.52
34	79.30 ± 2.50	86.29 ± 1.47	4.02 ± 1.92	5.53 ± 0.28
35	83.52 ± 1.54	75.19 ± 0.73	4.52 ± 0.56	8.29 ± 1.92
36	85.47 ± 0.28	70.13 ± 2.94	10.23 ± 0.95	6.31 ± 1.94
37	45.26 ± 0.57	68.27 ± 1.39	6.94 ± 0.68	7.51 ± 1.13
38	55.60 ± 1.62	72.03 ± 2.82	14.59 ± 0.77	16.36 ± 0.85
39	51.49 ± 3.02	75.61 ± 1.12	14.40 ± 0.58	15.27 ± 0.72
40	47.29 ± 1.16	65.93 ± 1.80	5.21 ± 0.82	6.23 ± 0.55
41	48.20 ± 0.47	74.70 ± 2.11	17.36 ± 0.56	16.22 ± 1.83

[a]化合物进行了三次重复试验。数据以平均值±SD表示。

[b]IC_{50}值由细胞活力测定24小时。

[0086] 实验结果表明,本发明的化合物均对BRD4和CK2具有抑制活性及抑制三阴性乳腺癌细胞增殖活性,其中,化合物31,32,33,34,35的效果较好,最优化合物32(44e)对BRD4和CK2具有较强的抑制活性及三阴性乳腺癌细胞抑制活性。

[0087] 试验例2化合物32(44e)在体外诱导MDA-MB-231细胞自噬,促进细胞死亡和凋亡研究

[0088] 对MDA-MB-231细胞进行GFP-mRFP-LC3腺病毒转染,通过倒置显微镜观察44e对细胞的影响。能够明显观察到化合物44e诱导MDA-MB-231细胞的自噬通量,说明其能够在MDA-MB-231细胞中诱导自噬(图1A)。

[0089] 克隆形成实验结果表明,44e在0μM,2.5μM,5μM和10μM浓度下,以浓度依赖性方式显着抑制MDA-MB-231细胞的生长和克隆形成(图1B)。

[0090] 图1C使用不同浓度的化合物44e处理MDA-MB-231细胞,化合物和细胞作用24h后,使用Hoechst 33258荧光染料对两种细胞进行染色,明显观察到细胞死亡数量以剂量依赖性方式显着增加(图1C)。

[0091] 使用流式细胞术测定细胞的凋亡率,化合物44e在0μM和5μM浓度下,能够以浓度依赖的方式诱导MDA-MB-231细胞的凋亡(图1D)。

[0092] 试验例3化合物32(44e)在体内治疗三阴性乳腺癌的效果评价

[0093] 建立MDA-MB-468和MDA-MB-231移植瘤模型并随机分为4组,每组6只,并进行连续19天的灌胃给药(Intragastric administration)方式。两种剂量的44e和药物连用组均在测试的剂量下表现出剂量依赖性的肿瘤生长抑制(TGI)作用(图2A,2B)。特别是在MDA-MB-231移植瘤模型中,应用剂量为50mg/kg 44e时,抑制活性最强,生长抑制率为63.8%。

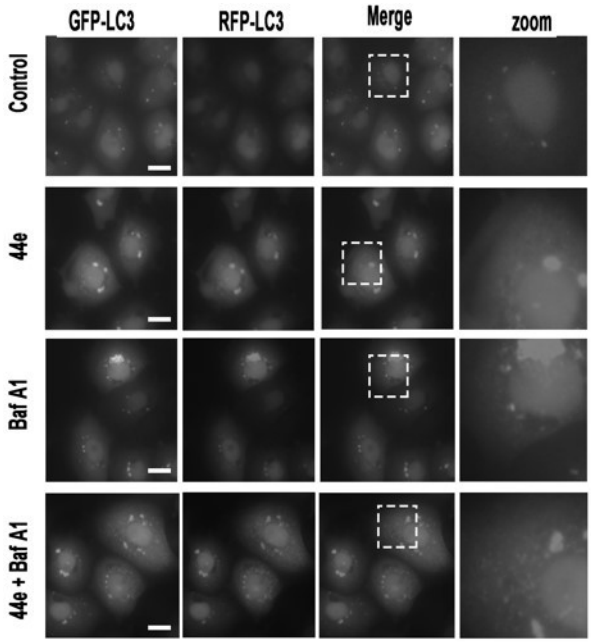
GFP-LC3
RFP-LC3
Merge
zoom
Control
44e
Baf A1
44e + Baf A1

图1A

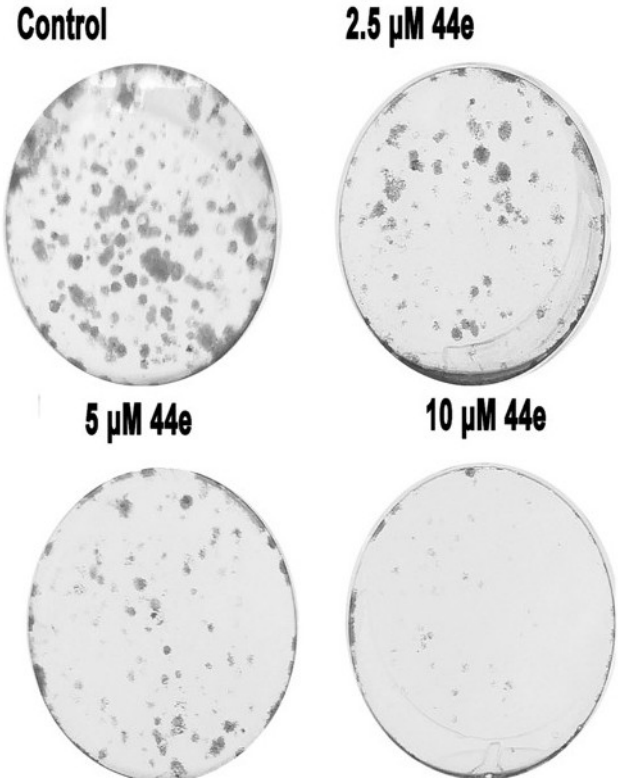
Control
2.5 μM 44e
5 μM 44e
10 μM 44e

图1B

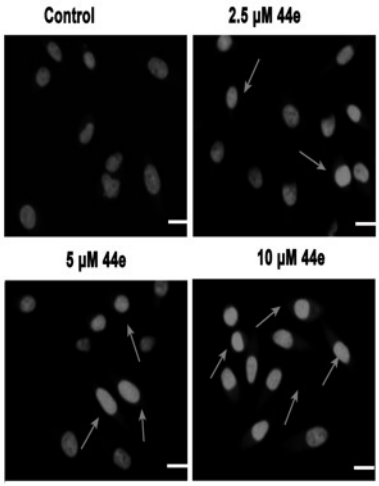
Control
2.5 μM 44e
5 μM 44e
10 μM 44e

图1C

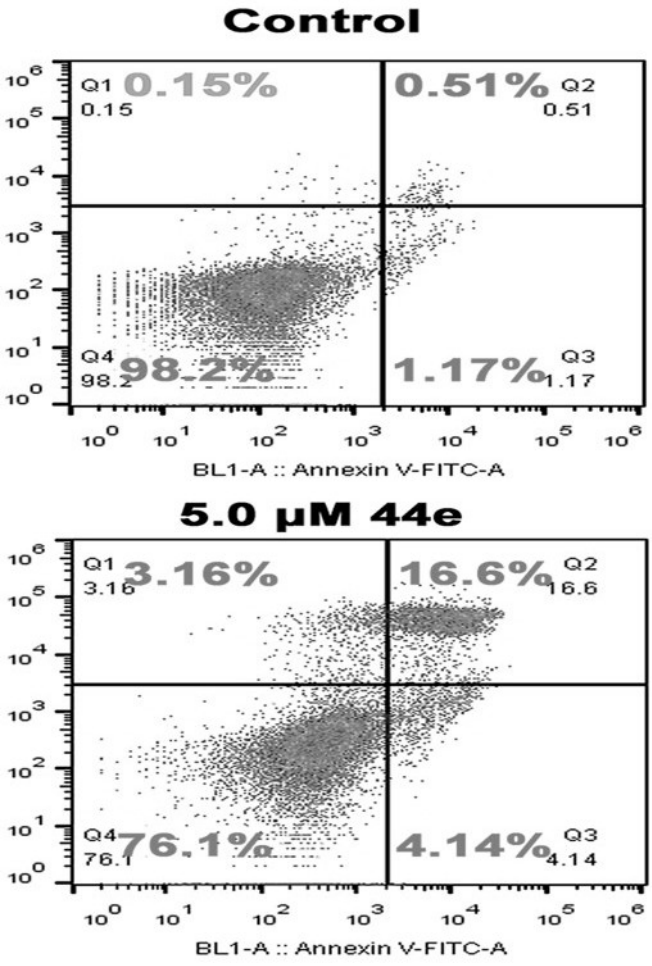
Control
Q1 0.15% 0.51% Q2
0.15 0.51
Q4 98.2% 1.17% Q3
98.2 1.17
BL1-A :: Annexin V-FITC-A
5.0 μM 44e
Q1 3.16% 16.6% Q2
3.16 16.6
Q4 76.1% 4.14% Q3
76.1 4.14
BL1-A :: Annexin V-FITC-A

图1D

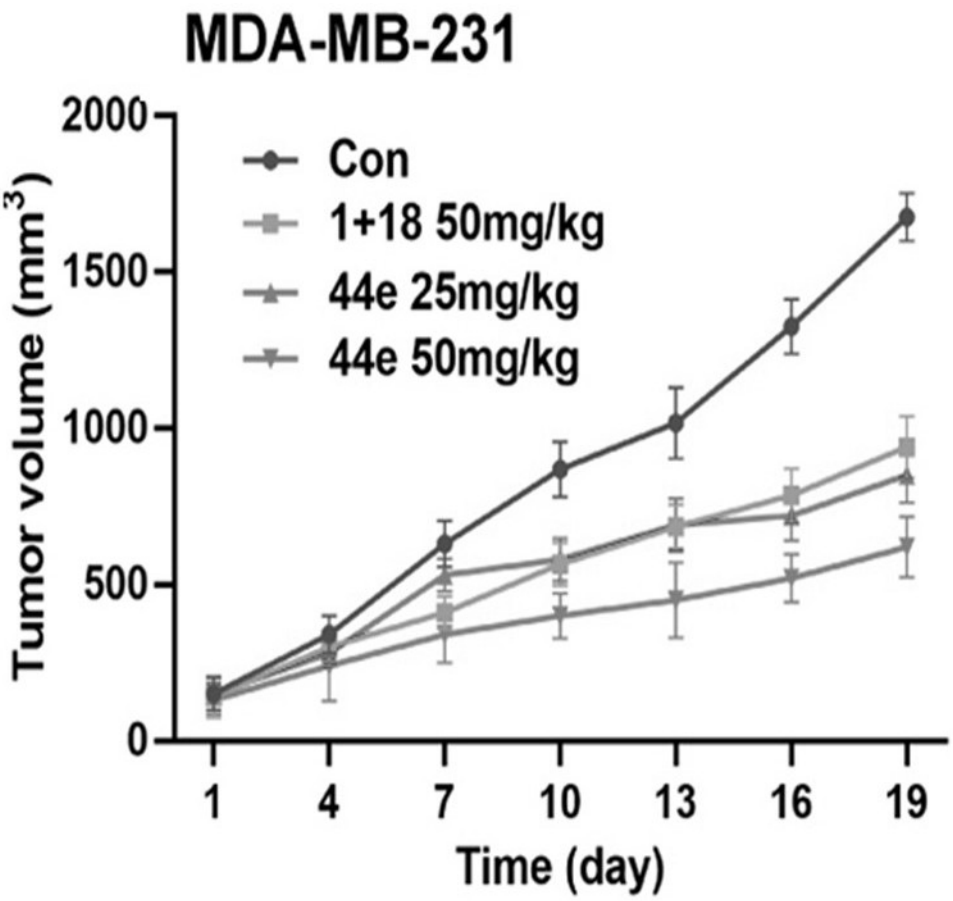
MDA-MB-231
Tumor volume (mm3)
2000
1500
1000
500
0
Con
1+18 50mg/kg
44e 25mg/kg
44e 50mg/kg
1
4
7
10
13
16
19
Time (day)

图2A

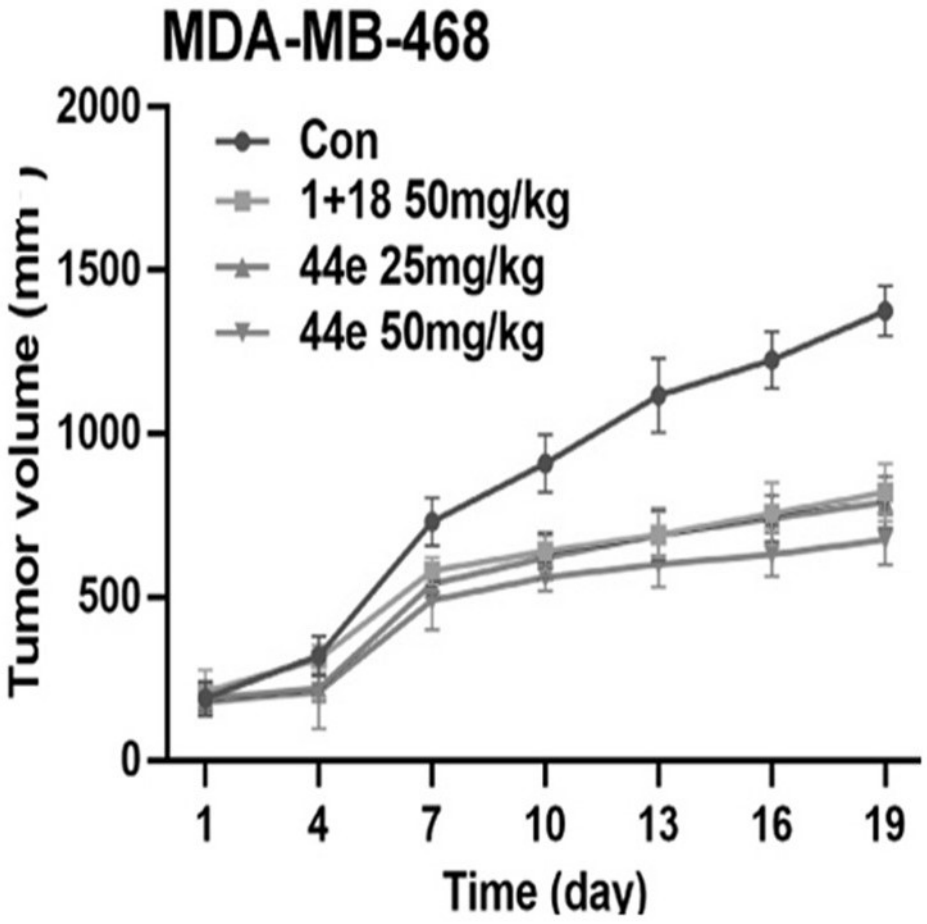
MDA-MB-468
Con
1+18 50mg/kg
44e 25mg/kg
44e 50mg/kg
Tumor volume (mm
2000
1500
1000
500
0
1
4
7
10
13
16
19
Time (day)

图2B